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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification: C07K 14/47, A61K 38/17, A61K 48/00, A61P 25/28, C12N 15/11, C12N 15/12, C12N 15/86, C12Q 1/68, G01N 33/53

A1

(11) International Publication Number: WO 00/46246

(43) International Publication Date: 10 August 2000 (10.08.2000)

(21) International Application Number: PCT/US00/02412

(22) International Filing Date: 02 February 2000 (02.02.2000)

(30) Priority Data:
09/241,606 02 February 1999 (02.02.1999) US

(60) Parent Application or Grant
THE GENERAL HOSPITAL CORPORATION [/];
(). TANZI, Rudolph, E. [/]; (). KOVACS, Dora, M. [/];
(). SAUNDERS, Aleister, J. [/]; (). TANZI, Rudolph, E. [/];
(). KOVACS, Dora, M. [/]; (). SAUNDERS, Aleister, J. [/];
(). GOLDSTEIN, Jorge, A. ; ().

Published

(54) Title: ALPHA-2-MACROGLOBULIN THERAPIES AND DRUG SCREENING METHODS FOR ALZHEIMER'S DISEASE

(54) Titre: THERAPIES A BASE D'ALPHA-2-MACROGLOBULINE ET PROCEDES DE CRIBLAGE DE MEDICAMENTS POUR LA MALADIE D'ALZHEIMER

(57) Abstract

The disclosed invention relates to the finding that the A2M-2 deletion mutation, which is a predisposing factor for Alzheimer's Disease, leads to the production of altered 'alpha'¿2M RNA transcripts and proteins. Based on this finding, the invention provides for new therapeutic agents for AD, including molecules having A'beta' and low density lipoprotein receptor-related protein (LRP) binding domains, peptides, nucleic acid molecules, antisense oligonucleotides, and viral vectors for gene therapy. In addition, the invention relates to pharmaceutical compositions containing these therapeutic agents, methods of using these therapeutic agents to combat Alzheimer's Disease, and methods of screening for therapeutic agents that can combat Alzheimer's Disease.

(57) Abrégé

On a découvert que la mutation par délétion de A2M-2, qui constitue un facteur de prédisposition à la maladie d'Alzheimer, conduisait à la production de transcrits et de protéines 'alpha'¿2M d'ARN modifiés. Ainsi, cette invention concerne des nouveaux agents thérapeutiques pour la maladie d'Alzheimer, dont des molécules présentant des domaines de liaison à l'A'beta' et à la protéine liée au récepteur de la lipoprotéine de faible densité (LRP), des peptides, des molécules d'acide nucléique, des oligonucléotides antisens, et des vecteurs viraux pour thérapie génique. L'invention porte également sur des compositions pharmaceutiques renfermant ces agents thérapeutiques, sur des méthodes d'utilisation desdits agents pour combattre la maladie Alzheimer et des procédés de criblage de tels agents.

[redacted]
ATTORNEY DOCKET NUMBER: 8449-134
SERIAL NUMBER: 09/750,972
REFERENCE: DV

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07K 14/47, C12N 15/12, 15/11, C12Q 1/68, A61K 48/00, C12N 15/86, G01N 33/53, A61K 38/17, A61P 25/28

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(11) International Publication Number: **WO 00/46246**

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(21) International Application Number: PCT/US00/02412

(22) International Filing Date: 2 February 2000 (02.02.00)

(30) Priority Data:
09/241,606 2 February 1999 (02.02.99) US

(71) Applicant *(for all designated States except US)*: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: TANZI, Rudolph, E. [US/US]; 3 Oceanside Drive, Hull, MA 02045 (US). KOVACS, Dora, M. [IT/US]; 65 East India Row, Apt. 23II, Boston, MA 02110 (US). SAUNDERS, Aleister, J. [US/US]; 35 Tufts Steet, #2, Cambridge, MA 02139 (US).

(74) Agents: GOLDSTEIN, Jorge, A. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., 1100 New York Avenue, N.W., Suite 600, Washington, DC 20005-3934 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

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(57) Abstract

The disclosed invention relates to the finding that the *A2M-2* deletion mutation, which is a predisposing factor for Alzheimer's Disease, leads to the production of altered α_2M RNA transcripts and proteins. Based on this finding, the invention provides for new therapeutic agents for AD, including molecules having Aβ and low density lipoprotein receptor-related protein (LRP) binding domains, peptides, nucleic acid molecules, antisense oligonucleotides, and viral vectors for gene therapy. In addition, the invention relates to pharmaceutical compositions containing these therapeutic agents, methods of using these therapeutic agents to combat Alzheimer's Disease, and methods of screening for therapeutic agents that can combat Alzheimer's Disease.

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Description

Alpha-2-Macroglobulin Therapies and Drug Screening Methods for Alzheimer's Disease

Background of the Invention

Field of the Invention

This invention relates to the field of medical genetics. More specifically, the invention provides for therapeutic agents for Alzheimer's Disease and methods of screening for therapeutic agents for Alzheimer's disease that are based on affecting alpha-2-macroglobulin function and expression.

Related Art

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that affects more than 4 million people per year in the US (Döbeli, H.; *Nat. Biotech. 15:* 223-24 (1997)). It is the major form of dementia occurring in mid to late life: approximately 10% of individuals over 65 years of age, and approximately 40% of individuals over 80 years of age, are symptomatic of AD (Price, D. L., and Sisodia, S. S., *Ann. Rev. Neurosci. 21:*479-505 (1998)).

The first recognized clinical symptom of AD is usually the loss of short-term memory (Schellenberg, G.D., *Proc. Natl. Acad. Sci. USA 92:*8552-559 (1995)). Other common symptoms include abnormal judgement and behavior, and difficulty with language, orientation, problem solving, calculations, and visuospacial perception (Price, D. L., and Sisodia, S. S., *Ann. Rev. Neurosci. 21:*479-505 (1998); Schellenberg, G.D., *Proc. Natl. Acad. Sci. USA 92:*8552-559 (1995)). These symptoms often worsen until cognitive function is almost entirely lost, and the patient cannot function independently (Schellenberg, G.D., *Proc. Natl. Acad. Sci. USA 92:*8552-559 (1995); Price, D. L., and Sisodia, S. S., *Ann. Rev. Neurosci. 21:*479-505 (1998)). By late stages of the disease patients typically lack verbal ability, cannot recognize people, and are incontinent and bed-ridden (Price, D. L., and Sisodia, S. S., *Ann. Rev. Neurosci. 21:*479-505 (1998); Sloane, P. D., *Am. Family Phys. 58:* 1577-86 (1998)).

Known risk factors for AD include age, genetic predisposition, abnormal protein (β-amyloid) deposition in the brain, and certain environmental factors such as head injury, hypothyroidism, and a history of depression. The majority of AD patients do not exhibit symptoms until their seventies (Price, D. L., and Sisodia, S. S., *Ann. Rev. Neurosci. 21*:479-505 (1998)). However, individuals who have inherited particular genetic defects often exhibit symptoms in midlife (Price, D. L., and Sisodia, S. S., *Ann. Rev. Neurosci. 21*:479-505 (1998)). This latter type of AD, called early-onset familial AD (FAD), accounts for 5-10% of AD cases, and has been linked to defects in three different genes, *APP*, *PSEN1*, *PSEN2* (Blacker, D. and Tanzi, R. E., *Archives of Neurology 55*:294-296 (1998)). Mutations in these genes lead to increased production of the amyloidogenic β-amyloid peptide (Aβ) (Citron, M., *et al., Nature Medicine 3*:67-72 (1997); Suzuki, N., *et al., Science 264*:1336-1340 (1994)).

The most prevalent form of AD, called late-onset AD (LOAD), accounts for approximately 90% of AD cases, and has been genetically linked to *APOE* and *LRP* (Kang, D. E., *et al., Neurology 49*:56-61 (1997); Kounnas, M. Z., *et al., Cell 82*:331-340 (1995)). Recently, another gene, the alpha-2-macroglobulin gene (*A2M*), was found to be linked to LOAD (Blacker, D., *et al., Nature Genetics 19*:357-360 (1998)). Carriers of a particular mutation in *A2M* were discovered to be at increased risk of AD. This mutation is a pentanucleotide deletion at the 5' splice site of the second exon encoding the bait region of alpha-2-macroglobulin (α_2M), and is referred to as the *A2M-2* genotype. The *A2M-2* genotype is present in 30% of the population (Blacker, D., *et al., Nature Genetics 19*:357-360 (1998)). The *A2M-2* pentanucleotide deletion is a predisposing factor for AD.

Presently, there is no cure for AD on the horizon and its incidence is increasing as the population ages (Price, D. L., and Sisodia, S. S., *Ann. Rev. Neurosci. 21*:479-505 (1998)). Due to the lateness in life of the onset of AD symptoms, the ability to delay onset by as little as 5 years could decrease the number of AD patients by as much as 50% (Marx, J., *Science 273*:50-53 (1996)). With the large number of people already affected, and projected to be affected by AD, a drug that could merely delay the onset of AD would be very valuable.

Therapeutic agents based on predisposing factors of AD might be able to prevent, delay or slow progression of the disease. However, presently, available treatments are primarily aimed at treatment of the symptoms of the disease (Enz, A., "Classes of drugs," in: Pharmacotherapy of Alzheimer's Disease, Gauthier, S., ed., Martin Dunitz, publ., Malden, MA (1998)). These AD drugs offer only modest success, and at most, merely slow the progression of the disease (Delagarza, V. W., *Am. Family Phys. 58:*1175-1182 (1998); Enz, A., "Classes of drugs," in: Pharmacotherapy of Alzheimer's Disease, Gauthier, S., ed., Martin Dunitz, publ., Malden, MA (1998)). Presently approved and investigational drugs for treating AD can be characterized as those whose actions enhance neurotransmitter effect, or those believed to protect neurons (Delagarza, V., *Am. Family Phys. 58:*1175-1182 (1998)). The most well known drugs in the first category are the cholinesterase inhibitors, such as tacrine (Cognex™) and doneprezil (Aricept™), both of which have been approved by the FDA (Delagarza, V., *Am. Family Phys. 58:*1175-1182 (1998); Sloan, P., *Am. Family Phys. 58:*1577-1586 (1998)). Tacrine and doneprezil are only modestly effective (Sloan, P., *Am. Family Phys. 58:*1577-1586 (1998)), and are associated with unpleasant side effects including nausea and vomiting (Delagarza, V., *Am. Family Phys. 58:*1175-1182 (1998)). Several neuro-protective drugs are under investigation for the treatment of AD, including estrogen, vitamin E, selegiline and non-steroidal anti-inflammatory drugs (NSAIDs) (Sloan, P., *Am. Family Phys. 58:*1577-1586 (1998); Delagarza, V., *Am. Family Phys. 58:*1175-1182 (1998)). None of these drugs have been approved yet for the treatment of AD, and each has significant drawbacks, including negative side-effects, or association with increased risk of other diseases. (Sloan, P., *Am. Family Phys. 58:*1577-1586 (1998); Delagarza, V., *Am. Family Phys. 58:*1175-1182 (1998); Enz, A., "Classes of drugs," in: Pharmacotherapy of Alzheimer's Disease, Gauthier, S., ed., Martin Dunitz, publ., Malden, MA (1998)).

Thus, there is a need for new AD therapeutic agents, especially those based on predisposing factors of AD. In addition, there is a need for drug screening systems to aid in developing these therapeutic agents.

Summary of the Invention

Based on the finding, described herein, that the *A2M-2* deletion leads to the production of altered α_2M RNA transcripts and proteins, strategies aimed at replacing or supplementing normal α_2M function and activities, and/or at suppressing defective α_2M function in the brain may serve as a means for therapeutically preventing, treating, or even reversing AD neuropathogenesis. In addition, these strategies may be useful for treating other pathologies associated with defective α_2M function. Moreover, methods described herein may be used to screen for these therapeutic agents. Thus, the invention provides for new therapeutic agents for AD, for pharmaceutical compositions containing these therapeutic agents, for methods of using these therapeutic agents, and for methods of screening for these therapeutic agents.

The first aspect of the invention is to provide for a therapeutic agent for Alzheimer's Disease, where the agent can replace or supplement α2M function, or can suppress the expression of *A2M-2*. A molecule that can bind to Aβ and to LRP may be able to promote clearance of Aβ through LRP mediated endocytosis. Thus, one embodiment of the invention is an anti-LRP-Aβ molecule having an Aβ binding domain, and an LRP binding domain. In a preferred embodiment of the invention, this molecule is a peptide.

In one embodiment of the invention the peptide is an anti-LRP-Aβ peptide having an Aβ binding domain composed of 10-50 contiguous residues of SEQ ID NO:6, and an LRP binding domain comprising 10-50 contiguous residues of SEQ ID NO:8, which encompass residues 1366-1392 of SEQ ID NO:8. In another embodiment of the invention, the anti-LRP-Aβ peptide has an Aβ binding domain with an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26; and an LRP binding domain composed of the amino acid sequence of SEQ ID NO:10. In yet another embodiment of the invention, the anti-LRP-Aβ peptide has an Aβ binding domain with an amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ

ID NO:24, and SEQ ID NO:26; and an LRP binding domain composed of 10-50 contiguous residues of SEQ ID NO:8.

The Aβ binding domain may be connected to the LRP binding domain of the anti-LRP-Aβ molecule by a covalent bond, linker molecule, or linkerless polyethylene glycol. In a preferred embodiment, the Aβ and LRP binding domains are connected by a peptide bond. In another preferred embodiment of the invention, the Aβ and LRP binding domains are connected by a peptide composed of 1-20 glycine residues.

In another embodiment, the anti-LRP-Aβ peptide has the amino acid sequence of SEQ ID NO:14. Alternatively, the anti-LRP-Aβ peptide has an Aβ binding domain having an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26; an LRP binding domain having the amino acid sequence of SEQ ID NO:10; and a linker connecting the Aβ binding domain to the LRP binding domain.

In addition, the invention provides for pharmaceutically acceptable salts of the anti-LRP-Aβ peptide and for nucleic acid molecules encoding the anti-LRP-Aβ peptide.

Another embodiment of the invention relates to a nucleic acid molecule encoding an anti-LRP-β peptide, where the Aβ binding domain is encoded by 30-150 contiguous nucleotides of SEQ ID NO:5, and the LRP binding domain is encoded by 30-150 contiguous nucleotides of SEQ ID NO:7. In another embodiment of the invention, the region of the nucleic acid molecule encoding the Aβ binding domain has a nucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25; and the region encoding the LRP binding domain has the nucleotide sequence of SEQ ID NO:9. In yet another embodiment of the invention, the region of the nucleic acid molecule encoding the Aβ binding domain has a nucleotide sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25; and the

region encoding the LRP binding domain is encoded by 30-150 contiguous nucleotides of SEQ ID NO:7. In another embodiment of the invention, the nucleic acid molecule has the nucleotide sequence of SEQ ID NO:13.

The region encoding the Aβ binding domain may be connected to the region encoding the LRP binding domain of the nucleic acid molecule by a phosphodiester bond. Alternatively, these regions may be connected by a nucleotide encoding a linker peptide. In a preferred embodiment of the invention, the connecting nucleotide encodes 1-20 glycine residues.

In addition, the invention relates to nucleic acid molecules having at least 95% homology to these nucleic acid molecules.

Another embodiment of the invention relates to a nucleic acid molecule that is a first polynucleotide that hybridizes to a second polynucleotide that is complementary to the nucleic acid molecules described above. In another embodiment of the invention, the nucleic acid molecule is a first polynucleotide that hybridizes to a second polynucleotide that is complementary to the nucleotide sequence of SEQ ID NO:13. In yet another embodiment of the invention, the hybridizing conditions for the hybridization of the first and second polynucleotides are as follows: (a) incubate overnight at 42°C in a solution consisting of 50% formamide, 5x SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and a 20 μg/ml denatured, sheared salmon sperm DNA; and (b) wash at 65°C in a solution consisting of 0.1x SSC.

A related embodiment of the invention is a pharmaceutical composition containing an anti-LRP-Aβ molecule, and one or more pharmaceutically acceptable carriers. In addition, the invention provides for a pharmaceutical composition containing an anti-LRP-Aβ peptide, or a pharmaceutically acceptable salt thereof. In a preferred embodiment, the pharmaceutical composition contains an anti-LRP-Aβ peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4 or SEQ ID NO:14, or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable carriers. The invention also relates to a method of combating Alzheimer's Disease in a subject by administering an anti-LRP-Aβ molecule, or a pharmaceutically acceptable salt

thereof. In a preferred embodiment, the anti-LRP-Aβ molecule is a peptide. In another preferred embodiment, the anti-LRP-Aβ peptide is a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4 or SEQ ID NO:14, or a pharmaceutically acceptable salt thereof.

The invention also relates to an *A2M-2* antisense oligonucleotide designed to target *A2M-2* RNA. In one preferred embodiment of the invention, the *A2M-2* antisense oligonucleotide is designed to target *A2M-2* heteronuclear RNA. In another preferred embodiment, the *A2M-2* antisense oligonucleotide is designed to target *A2M-2* mRNA. In one embodiment of the invention, the *A2M-2* antisense oligonucleotide designed to target *A2M* hnRNA has the nucleotide sequence of SEQ ID NO:27. The *A2M-2* antisense oligonucleotide is preferably from 8- 50 nucleotides in length, and more preferably is 15-30 nucleotides in length, and is most preferably 15 nucleotides in length. Thus, in another preferred embodiment of the invention an *A2M-2* antisense oligonucleotide designed to target *A2M-2* hnRNA has the nucleotide sequence of the last 15-30 contiguous nucleotides of SEQ ID NO:27. In another embodiment of the invention the *A2M-2* antisense oligonucleotide designed to target *A2M-2* has the sequence of nucleotides 36-50 of SEQ ID NO:27 or of nucleotides 20 -50 of SEQ ID NO:27. The invention also relates to a pharmaceutical composition containing an *A2M-2* antisense oligonucleotide, and one or more pharmaceutically acceptable carriers. In addition, the invention relates to a method of combating Alzheimer's Disease in a subject by administering the *A2M-2* antisense oligonucleotide.

The invention also provides for a viral vector carrying a transgene encoding α_2M, or an anti-LRP-Aβ peptide. In a preferred embodiment of the invention, the viral vector carries a gene encoding α_2M. In another preferred embodiment of the invention, the gene encoding α_2M has the nucleotide sequence of nucleotides 44-4465 of SEQ ID NO:1. The invention also relates to a viral vector carrying a gene encoding an anti-LRP-Aβ peptide. In another preferred embodiment of the invention, the viral vector is an adeno-associated virus. In addition, the invention provides for a pharmaceutical composition containing the viral vector, and one or more pharmaceutically acceptable carriers, and for a

method of combating Alzheimer's Disease in a subject by administering the viral vector.

The second aspect of the invention is to provide for a method of screening for therapeutic agents for Alzheimer's Disease that can replace or supplement α2M function, or can suppress the expression of *A2M-2*. One embodiment of the invention is a method of screening for a therapeutic agent for AD by incubating a cell that is heterozygous or homozygous for the *A2M-2* allele in the presence of a test agent, and then determining whether the ratio of normal to aberrant *A2M* mRNA has increased relative to the ratio of normal to aberrant *A2M* mRNA found in cells untreated with the test agent. In one preferred embodiment of this method, the cells are glioma cells. In another preferred embodiment, the cells are hepatoma cells. In yet another preferred embodiment of the invention, the cells are heterozygous for the *A2M-2* allele.

In a related embodiment of this method, S1 nuclease is used to determine the ratio of normal to aberrant *A2M* mRNA, and the probe used is complementary to a nucleotide encoding *A2M* (SEQ ID NO:1). Thus, in one embodiment of the invention, S1 nuclease analysis using a probe complementary to SEQ ID NO:1, where the probe encompasses nucleotides 2057-2284 of SEQ ID NO:1, is used to determine whether the ratio of normal to aberrant *A2M* mRNA has increased. In a preferred method of the invention, the probe used in the S1 nuclease analysis is 300 bp long. In another embodiment of the invention, the probe used in the S1 nuclease analysis is complementary to nucleotides 2024-2323 of SEQ ID NO:1.

Alternatively, RT PCR analysis is used to determine whether the ratio of normal to aberrant *A2M* mRNA has increased. In a preferred method of RT PCR analysis, the primers are designed to amplify a region of *A2M* encompassing exons 17-18. In a more preferred method of RT PCR analysis, the amplified region of *A2M* encompassing exons 17-18 is 300 bp long. In another embodiment of the invention, the primers used for the RT PCR analysis are designed to amplify nucleotides 2052-2289 of SEQ ID NO:1. Another embodiment of the invention relates to the use of a first primer having a nucleotide sequence complementary to nucleotides 2024-2038 of SEQ ID NO:1, and a second primer having the

nucleotide sequence of nucleotides 2309-2323 of SEQ ID NO:1 for the RT PCR analysis.

The invention also provides for a method of screening for a therapeutic agent for Alzheimer's disease by incubating α_2M with a test agent, and then determining whether the treated α_2M has undergone a conformational change, or determining whether the treated α_2M can bind to LRP. In a preferred embodiment of the invention, the α_2M treated with a test agent is tetrameric α_2M. In another preferred embodiment of the invention, an α_2M electrophoretic mobility assay is ued to determine whether the treated α_2M has undergone a conformational change. In another embodiment of the invention, an ELISA is used to determine whether the treated α_2M can bind to LRP. In a related embodiment of the invention, the ELISA includes the following steps in sequential order: incubating LRP in a well coated with anti-LRP IgG, incubating the well with treated α_2M, incubating the well with anti-α_2M IgG conjugated to an enzyme, and incubating the well with a substrate for the enzyme. In an alternative embodiment, the ELISA includes the following steps in sequential order: incubating a well coated with LRP with treated α_2M, incubating the well with anti-α_2M IgG conjugated to an enzyme, and incubating the well with the substrate for the enzyme. In another embodiment, the ELISA includes the following steps in sequential order: incubating treated α_2M in a well coated with an anti-α_2M IgG specific for activated α_2M, incubating the well with an anti-α_2M IgG conjugated to an enzyme, and incubating the well with a substrate for the enzyme. In another embodiment of the invention, immunoblotting with anti-LRP IgG and anti-α_2M IgG is used to determine whether the treated α_2M can bind to LRP. In yet another embodiment of the invention, a test for the ability of the treated α_2M to undergo LRP mediated endocytosis is used to determine whether the treated α_2M can bind to LRP. In another embodiment of the invention, a test for the ability of the treated α_2M to undergo LRP mediated degradation is used to determine whether the treated α_2M can bind to LRP.

Brief Description of the Figures

Figure 1. Figure 1 is an autoradiograph depicting the results of ^{33}P-labeled α_2M mRNA transcripts from *A2M* from human glioma cell lines that express either wild-type *A2M* ((Blacker, D., *et al.*, *Nat. Genet. 19*:357-360 (1998)) or are heterozygous for the *A2M-2* deletion allele obtained by RT-PCR, and separated on a polyacrylamide gel. *A2M*-1/2 lines are indicated as lanes marked "2", *A2M*-1/1 lines are indicated as lanes marked "1."

Figure 2. Figure 2 is a schematic representation of four of the altered *A2M* transcripts produced by human glioma cell lines expressing the *A2M-2* allele.

Figure 3. Figure 3 is a photograph of immunoblots of media and extracts from CHO cells transfected with α_2M truncated after exon 18 that were probed with an anti-α_2M antibody. The anti-α_2M antibody detected truncated α_2M in transfected CHO cells. Panel A: cell lysate; Panel B: media; (-) indicates samples from untransfected cells; (wt) indicates samples from cells transfected with full-length α_2M construct; (Δ) indicates samples from cells transfected with the α_2M construct truncated after exon 18; m, d and t indicate monomer, dimer and trimer forms of the truncated protein, respectively. These forms of wild type α_2M are also visible but not marked.

Figure 4. Figure 4 is a photograph of an immunoblot from cell lysates from wild-type cells (*A2M-1*) (lane labeled 1/1) and cells heterozygous for the *A2M-2* deletion (lanes labeled 1/2) probed with an anti-α_2M antibody. The lane labeled (+) indicates lysate from CHO cells transfected with full length α_2M, and probed with an anti-α_2M antibody. The media (data not shown) from *A2M-1* and *A2M-2* cells contained primarily full-length α_2M monomers, but in the media from the *A2M-2* cells, small amounts of truncated species could also be observed (data not shown).

Figure 5. Figure 5 depicts the α_2M conformational change induced by protease (represented by the letter P in a circle) cleavage. Note the exposure of the LRP binding domain (represented by ⊐) after the conformational change.

Figure 6. Figure 6 depicts one possible amino acid sequence for the anti-LRP-Aβ polypeptide.

Figure 7. Figure 7 is a schematic of the yeast three-hybrid system for detecting the anti-LRP-Aβ peptide binding to Aβ and LRP.

Detailed Description of the Preferred Embodiments

Definitions

In the description that follows, a number of terms used in recombinant DNA technology, molecular and cell biology, and pharmacology are extensively used. To provide a clearer and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Nucleotide: "Nucleotide" refers to a base-sugar-phosphate combination. Nucleotides are monomeric units of a nucleic acid sequence (DNA and RNA). The term nucleotide includes deoxyribonucleoside triphosphates such as dATP, dCTP, dITP, dUTP, dGTP, dTTP, or derivatives thereof. Such derivatives include, for example, [αS]dATP, 7-deaza-dGTP and 7-deaza-dATP. The term nucleotide as used herein also refers to dideoxyribonucleoside triphosphates (ddNTPs) and their derivatives. Illustrated examples of dideoxyribonucleoside triphosphates include, but are not limited to, ddATP, ddCTP, ddGTP, ddITP, and ddTTP. According to the present invention, a "nucleotide" may be unlabeled or detectably labeled by well known techniques. Detectable labels include, for example, radioactive isotopes, fluorescent labels, chemiluminescent labels, bioluminescent labels and enzyme labels.

Polynucleotide: A "polynucleotide" is a linear polymer of nucleotides linked by phosphodiester bonds between the 3' position of one nucleotide and the 5' position of the adjacent nucleotide.

Oligonucleotide: "Oligonucleotide" refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars and intersugar (backbone) linkages. The term "oligonucleotide" also includes oligomers comprising non-naturally occurring monomers, or portions thereof, which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms in that they may exhibit enhanced cellular uptake,

increased stability in the presence of nucleases, and other features which render them more acceptable as therapeutic or diagnostic reagents.

Nucleic acid molecule: By "nucleic acid molecule" is meant a polymeric molecule composed of nucleotides. Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

Complementary: As used herein, "complementary" refers to the subunit sequence complementarity between two nucleic acids, for example, two DNA molecules. When a nucleotide position in both of the molecules is occupied by nucleotides normally capable of base pairing with each other, then the nucleic acids are considered to be complementary to each other at this position. Thus, two nucleic acids are complementary to each other when a substantial number (at least 60%) of corresponding positions in each of the molecules are occupied by nucleotides which normally base pair with each other (for example, A:T and G:C nucleotide pairs).

Hybridization: The terms "hybridization" and "specifically hybridizes to" refer to the pairing of two complementary single-stranded nucleic acid molecules (RNA and/or DNA) to give a double-stranded molecule. These terms are used to indicate that the nucleotides are sufficiently complementary such that stable and specific binding occurs between the DNA or RNA target and the oligonucleotide. It is understood that an oligonucleotide need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable. An oligonucleotide specifically hybridizes to another when binding of the oligonucleotide to the target interferes with the normal function of the target molecule to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, *i.e.*, under physiological conditions in the case of *in vivo* assays

or therapeutic treatment, or, in the case of *in vitro* assays, under conditions in which the assays are conducted.

Primer: As used herein "primer" refers to a single-stranded oligonucleotide that is extended by covalent bonding of nucleotide monomers during amplification or polymerization of a DNA molecule. Minisatellite primers used for the amplification of minisatellite dimer, trimer, tetramer, etc., sequences are well-known in the art.

Template: The term "template" as used herein refers to a double-stranded or single-stranded nucleic acid molecule which is to be amplified, synthesized or sequenced. In the case of a double-stranded DNA molecule, denaturation of its strands to form a first and a second strand is performed before these molecules may be amplified, synthesized or sequenced. A primer, complementary to a portion of a DNA template is hybridized under appropriate conditions and the DNA polymerase of the invention may then synthesize a DNA molecule complementary to the template or a portion thereof. The newly synthesized DNA molecule, according to the invention, may be equal or shorter in length than the original DNA template. Mismatch incorporation or strand slippage during the synthesis or extension of the newly synthesized DNA molecule may result in one or a number of mismatched base pairs. Thus, the synthesized DNA molecule need not be exactly complementary to the DNA template.

Amplification: As used herein "amplification" refers to any *in vitro* method for increasing the number of copies of a nucleotide sequence with the use of a DNA polymerase. Nucleic acid amplification results in the incorporation of nucleotides into a DNA or molecule or primer thereby forming a new DNA molecule complementary to a DNA template. The formed DNA molecule and its template can be used as templates to synthesize additional DNA molecules. As used herein, one amplification reaction may consist of many rounds of DNA replication. DNA amplification reactions include, for example, polymerase chain reactions (PCR). One PCR reaction may consist of 5 to 100 "cycles" of denaturation and synthesis of a DNA molecule.

95%, 96%, 97%, 98% or 99% Homology: By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5′ or 3′ terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in SEQ ID NO:1 can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711. Bestfit uses the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics 2*: 482-489 (1981), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

Polypeptide: A polypeptide is a polymer composed of amino acid monomers joined by peptide bonds.

Peptide Bond: A peptide bond is a covalent bond between two amino acids in which the alpha-amino group of one amino acid is bonded to the alpha-carboxyl group of the other amino acid.

Isolated nucleic acid molecule or polypeptide: a nucleic acid molecule, DNA or RNA, or a polypeptide, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules or polypeptides according to the present invention further include such molecules produced synthetically.

Linker: By "linker" is intended a molecule that connects the LRP binding domain to the Aβ binding domain of the anti-LRP-Aβ molecule. When referring to a linker composed of amino acid residues, linker is used to refer to the amino acid residues connecting the two domains. When referring to a nucleic acid encoding a linker, linker refers to the nucleotide sequence encoding the linking amino acid residues. Where the linker is composed of amino acid residues, it will typically consist of one or more glycine residues, or the nucleotide sequence encoding these residues, however, proline may also be used.

Combating Alzheimer's Disease: The term "combating Alzheimer's Disease" is intended to mean a slowing, delaying, or even reversing the AD process. Thus, for example, the therapeutic agents of the invention may be administered either therapeutically in a patient where symptoms of AD are present, or prophylactically, in a subject at risk of developing AD.

Pharmaceutically acceptable carrier: By pharmaceutically acceptable carrier is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material, or formulation auxiliary of any type.

Performed in sequential order: By "performed in sequential order" is intended that the steps described by this term are performed in the order that the

steps are recited, but that other unrecited steps may be performed in between the recited steps.

Test agent: By "test agent" is meant any molecule that is of interest for the treatment or prevention of AD, and is to be tested using the screening methods of the invention.

Ranges: various ranges of numbers are described herein. When a range is used, the range of numbers is meant to be inclusive of the boundary numbers. For example, an oligonucleotide composed of nucleotides 20-50 of SEQ ID NO:27, is meant to include nucleotides 20, and 50 and every nucleotide in between.

Other terms used in the fields of recombinant DNA technology, molecular and cell biology, and pharmacology as used herein will be generally understood by one of ordinary skill in the applicable arts.

Alpha-2-macroglobulin

Alpha-2-Macroglobulin (α_2M) is a 718 kD glycoprotein found at high concentrations in the serum (Borth, W., *FASEB J. 6*:3345-3353 (1992)). The structure of α_2M consists of four identical 180 kD monomeric units, of 1451 amino acids each (Sottrup-Jensen, L., *et al., J. Biol. Chem. 259*:8318-8327 (1984)). Disulfide bonds link these monomers into dimers, and noncovalent interactions between dimers lead to formation of the functional homotetramer (Harpel, P. C., *J. Exp. Med. 138*:508-521 (1973); Swenson, R. P. and Howard, J. B., *J. Biol. Chem. 254*:4452-4456 (1979)). In addition to the ability to bind Aβ, α_2M binds a variety of polypeptides (proteases, growth factors, and cytokines) and ions (Zn, Cu, Fe)(Borth, W., *FASEB J. 6*:3345-3353 (1992); James, K., *Immunol. Today 11*:163-166 (1990); Parisi, A. F. and Vallee, B. L., *Biochem. 9*:2421-2426 (1970)).

The best studied function of α_2M is its pan-protease inhibitory activity (Barret, A. J. and Starkey, P. M., *Biochem. J. 133*:709-724 (1973)). A protease molecule binds the bait region of a α_2M tetramer, amino acids 666-706, and cleaves any of a number of susceptible peptide bonds in this region ((Harpel, P.

C., *J. Exp. Med. 138*:508-521 (1973); Barret, A. J. and Starkey, P. M., *Biochem. J. 133*:709-724 (1973); Sottrup-Jensen, L., *et al., J. Biol. Chem. 264*:15781-15789 (1989)). Protease binding and cleavage triggers a large conformational change in the α_2M/protease complex, referred to as activation, that ultimately results in entrapment of the protease within the tetramer (Figure 5) (Borth, W., *FASEB J. 6*:3345-3353 (1992)). In each monomer a unique β-Cys-γ-Glu thiol ester bond exists between Cys-949 and Glu-952(Borth, W., *FASEB J. 6*:3345-3353 (1992)). Upon activation this thiol ester bond emerges from a hydrophobic environment and can undergo nucleophilic attack, for example, by lysine residues from the reacting proteases. The result of this nucleophilic attack is a covalent bond between Glu-952 of α_2M and surface lysine residues of the protease (Figure 5). The protease is effectively trapped, unable to dissociate from α_2M but still able to cleave small peptide substrates (Qui, W. Q., *et al., J. Biol. Chem. 271*:8443-8451 (1996)). Protease-mediated activation results in exposure of the α_2M receptor/low density lipoprotein receptor-related protein binding domain (Figure 5) (Strickland, D., *et al., J. Biol. Chem. 265*:17401-17404 (1990)). Low density lipoprotein receptor-related protein (LRP) is a 600 kD endocytic membrane-bound receptor belonging to the low-density lipoprotein receptor family (Borth, W., *FASEB J. 6*:3345-3353 (1992)). LRP is a multifunctional receptor, because it binds ligands from different classes (Kounnas, M. Z., *et al., Cell 82*:331-340 (1995)). Exposure of this LRP binding domain is a prerequisite for *LRP* mediated endocytosis of α_2M/ligand complexes and targeted degradation (Borth, W., *FASEB J. 6*:3345-3353 (1992)). In summary, α_2M serves to bind a number of protein substrates, including Aβ, and target them for internalization and degradation.

α_2M binds Aβ specifically and tightly. The Aβ binding region of α_2M is located between residues 1202-1312, approximately 600 residues C-terminal to the bait region (Hughes, S. R., *et al., Proc. Natl. Acad. Sci. USA 95*:3275-3280 (1998)). Binding does not require α_2M activation and binding stoichometry is approximately 1.1 Aβ / mol of α_2M (Du, Y., *et al., J. Neurochem. 69*:299-305 (1997)). The apparent dissociation constant (K_D) for the Aβ/α_2M complex has

been reported as $3.8 * 10^{-10}$ M for $\alpha_2M/^{125}I$-Aβ (Du, Y., *et al., J. Neurochem. 69*:299-305 (1997)) and $3.5 * 10^{-7}$ M for biotinAβ/(ruthenium (II) tris-bipyridine-n-hydroxysuccinimide ester) modified-α_2M (Hughes, S. R., *et al., Proc. Natl. Acad. Sci. USA 95*:3275-3280 (1998)). Despite this discrepancy in K_D values (which are most likely due to methodological differences), a strong interaction between Aβ and α_2M exists. This interaction prevents Aβ fibril formation and fibril associated neurotoxicity ((Hughes, S. R., *et al., Proc. Natl. Acad. Sci. USA 95*:3275-3280 (1998); Du, Y., *et al., J. Neurochem. 70*:1182-1188 (1998)). Recently, it has been demonstrated that a region of α_2M encompassing only the Aβ and *LRP* binding domains is sufficient for Aβ binding *in vivo* ((Hughes, S. R., *et al., Proc. Natl. Acad. Sci. USA 95*:3275-3280 (1998)). These data suggest that the Aβ binding domain is an independent structural unit and successful α_2M/Aβ interaction may only rely on a few key interactions. Recent work by Soto and colleagues show that an eleven residue peptide is capable of binding Aβ and inhibiting Aβ fibril formation (Soto, C., *et al., Nature Medicine 4*:822-826 (1998)), supporting the idea that only a few key interactions are needed to bind Aβ. In summary, α_2M can mediate the catabolism of Aβ in a *LRP* dependent process.

A2M-2 Genotype

The *A2M-2* genotype, which is linked to late-onset AD, is present in 30% of the population (Blacker, D., *et al., Nature Genetics 19*:357-360 (1998)). This genotype has a pentanucleotide deletion at the 5' splice site of the second exon encoding the bait region of α_2M (exon 18) (Blacker, D., *et al., Nature Genetics 19*:357-360 (1998)).

Low resolution X-ray data and biochemical data suggest that the bait regions are located at the dimer interface and are crucial for the formation of functional tetramers, and the mediation of the conformational change that accompanies activation (Andersen, G. R., *et al., J. Biol. Chem. 270*:25133-25141 (1995); Bowen, M. E. and Gettins, P. G. W., *J. Biol. Chem. 273*:1825-1831 (1998)). The *A2M-2* deletion in the bait region could prevent Aβ clearance and

degradation if (i) proteases can not cleave the altered bait region, (ii) protease-induced activation cannot occur, (iii) *LRP* binding is disrupted, and/or (iv) Aβ binding is disrupted.

Low density Lipoprotein Receptor-Related Protein

LRP is a 600 kD endocytic membrane-bound receptor belonging to the low-density lipoprotein receptor family (Borth, W., *FASEB J. 6*:3345-3353 (1992)). LRP is expressed in a variety of cell types including: adipocytes, astrocytes, fibroblasts, hepatocytes, macrophages, monocytes, and syncytiotrophoblasts. *LRP* is translated as a 4525 residue single chain precursor (Nielsen, K. L., *et al., J. Biol. Chem. 271*:12909-12912 (1996)). It is then processed into a 515 kD A chain and an 85 kD β chain. The β chain possesses a single transmembrane segment and a cytoplasmic tail containing two copies of the NPXY endocytosis signal sequence (Nielsen, K. L., *et al., J. Biol. Chem. 271*:12909-12912 (1996)). The extracellularly located α chain contains four cysteine-rich LDL receptor ligand-binding repeats flanked by epidermal growth factor (EGF) repeats (Nielsen, K. L., *et al., J. Biol. Chem. 271*:12909-12912 (1996)). The noncovalent association of the α chain with the extracellular portion of the β chain forms a functional LRP (Borth, W., *FASEB J. 6*:3345-3353 (1992)). LRP is a multifunction receptor because it binds ligands from different classes (Kounnas, M. Z., *et al., Cell 82*:331-340 (1995)). These include α_2M-protease complexes, plasminogen activator inhibitor-plasminogen activator complexes, lipoprotein lipase, apoE, bovine pancreatic trypsin inhibitor, lactoferrin, Pseudomonas, exotoxin A, nexin-1 complexes, and receptor associated protein (RAP) (Kounnas, M. Z., *et al., Cell 82*:331-340 (1995)). Most of these ligands do not compete for the same binding site. RAP, however, inhibits the binding of all these ligands.

α_2M/LRP Association

The association of activated α_2M and LRP is highly pH dependent, acidification to pH 6.8 or below abolishes binding (Borth, W., *FASEB J. 6*:3345-

3353 (1992)). This suggests that upon endocytosis α_2M dissociates from *LRP*. After endocytosis α_2M and its associated ligands are degraded in the lysosome and LRP is recycled to the membrane (Borth, W., *FASEB J. 6*:3345-3353 (1992)). The half-life for internalization and degradation varies between 15 and 60 minutes (Borth, W., *FASEB J. 6*:3345-3353 (1992)).

The α_2M-protease binding site of LRP has been mapped to residues 776-1399 of the β chain (Nielsen, K. L., *et al., J. Biol. Chem. 271*:12909-12912 (1996)). This region includes EGF repeats 4-6 and LDL receptor ligand binding repeats 3-10. The LRP binding domain of α_2M is located between residues 1312 and 1451, directly C-terminal to the Aβ binding domain (Hughes, S. R., *et al., Proc. Natl. Acad. Sci. USA 95*:3275-3280 (1998)). This domain is very flexible relative to the core of α_2M (Andersen, G. R., *et al., J. Biol. Chem. 270*:25133-25141 (1995)). Low resolution crystal structures (10Å) indicate that activated α_2M is roughly the shape of an H and the LRP binding domains are located at the tips of the H (Figure 5) (Andersen, G. R., *et al., J. Biol. Chem. 270*:25133-25141 (1995)). A LRP consensus binding sequence has been proposed based on 31 LRP ligands from 7 different protein families (Nielsen, K. L., *et al., J. Biol. Chem. 271*:12909-12912 (1996)). This 27 residue consensus sequence is located between residues 1365 and 1393 of human α_2M. Once again, experimental evidence suggests that a few key interactions may be important in LRP/α_2M binding. Mutations at positions 5 and 10 of the consensus sequence, corresponding to Lys-1370 and Lys-1374 in the human α_2M, abolish binding unlike mutations at other highly conserved residues.

Implication of α_2M in Alzheimer's Disease

Cerebral deposition of amyloid is a central event in AD (Soto, C., *et al., Nat. Med. 4*:822-826 (1998)). Genetic, neuropathological, and biochemical evidence indicate that inappropriate deposition of amyloid plays a fundamental role in the pathogenesis of AD. The major component of AD amyloid plaques is Aβ, a 39-43 amino acid peptide. Aβ polymerizes as dense (amyloid plaque) and diffuse extracellular deposits in the neuropil (Masters, C. L., *et al., Proc. Natl.*

Acad. Sci. USA 82:4245-4249 (1985)), and in cerebral blood vessels (congophilic angiopathy) (Glenner, G. G. and Wong, C. W., *Biochem. Biophys. Res. Comm. 120*:885-890 (1984)) of both AD and Down syndrome (DS) patients. Soluble Aβ is found in the cerebrospinal fluid (CSF) and is produced (Haass, C., *et al., Nature 359*:322-325 (1992); Seubert, P., *et al., Nature 359*:325-327 (1992); Shoji, M., *et al., Science 258*:126-129 (1992)) by constitutive cleavage of its transmembrane parent molecule, the amyloid protein precursor (APP) (Kang, J., *et al., Nature 325*:733-736 (1987); Goldbarger, D., *et al., Science 235*:877-880 (1987); Robakis, N. K., *et al., Proc. Natl. Acad. Sci. USA 84*:4190-4194 (1987); Tanzi, R. E., *et al., Science 235*:880-884 (1987)). APP is a family of alternatively-spliced proteins, of unknown function, that are ubiquitously expressed (Tanzi, R. E., *et al., Nature 331*:528-530 (1988)). Unknown proteases cleave APP to produce a mixture of Aβ peptides with carboxyl-terminal heterogeneity. Aβ1-40, the major soluble Aβ species, is found in the CSF at low nanomolar concentrations (Vigo-Pelfrey, C., *et al., J. Neurochem. 61*:1965-1968 (1993)). Aβ1-42 is a minor soluble Aβ species, but is heavily enriched in amyloid plaques (Masters, C. L., *et al., Proc. Natl. Acad. Sci. USA 82*:4245-4249 (1985); Kang, J., *et al., Nature 325*:733-736 (1987); Roher, A. E., *et al., J. Biol. Chem. 268*:3072-3083 (1993)).

The mechanism by which these amyloid deposits result in dementia is unclear, but may be related to the neurotoxic effects of Aβ at micromolar concentrations (Pike, C. J., *et al., Brain Res. 563*:311-314 (1991)). Insight into the mechanism of amyloid deposit formation began with the discovery of pathogenic mutations of APP close to, or within, the Aβ domain (van Broeckhoven, C., *et al., Science 248*:1120-1122 (1990); Levy, E., *et al., Science 248*:1124-1126 (1990); Goate, A., *et al., Nature 349*:704-706 (1991); Murrell, J., *et al., Science 254*:97-99 (1991); Mullan, M., *et al., Nat. Genet. 1*:345-347 (1992)). These studies indicated that the metabolism of Aβ, and APP, is intimately involved with the pathophysiology of AD. Increasing evidence suggests that increased levels of Aβ1-42 accelerates amyloid deposition in early-onset familial AD (FAD). The FAD-linked *APP*670/671 mutation has been shown to

increase the secretion of Aβ species several-fold (Citron, M., *et al., Nature 360*:672-674 (1992)). While the *APP*717 mutation does not affect the quantity of Aβ production (Cai, X-D., *et al., Science 259*:514-516 (1993)), this mutation increases the proportion of Aβ1-42 produced (Suzuki, N., *et al., Science 264*:1336-1340 (1994)). Increased soluble Aβ1-42 has also been found in the brains of individuals affected by Down syndrome, a condition complicated by premature AD (Teller, J. K., *et al., Nat. Med. 2*:93-95 (1996)). Inheritance of the other FAD-linked mutations of Presenilin-1 *(PSEN1)* or Presenilin-2 *(PSEN2)* (Sherrington, R., *et al., Nature 375*:754-760 (1995); Levy-Lahad, E., *et al., Science 269*:973-977 (1995)) correlates with increased cortical amyloid burden. The emerging consensus is that the common effect of FAD-linked presenilin mutations is to increase Aβ1-42 production (Citron, M., *et al., Nat. Med. 3*:67-72 (1997); Xia, W., *et al., J. Biol. Chem. 272*:7977-7982 (1997)). Taken together these studies suggest that mutations in the genes linked to FAD (*APP, PSEN1, PSEN2*) can result in increased Aβ1-42 production and that this increase could cause FAD. In the vast majority of AD patients, however, overproduction does not occur (Van Gool, W. A., *et al., Ann. Neurol. 37*:277-279 (1995)).

Ninety percent of AD patients suffer from late-onset AD (LOAD). Three genes have been linked to this form of AD:*APOE, LRP*, and *A2M*. Inheritance of the *APOE-ε4* allele on chromosome 19 correlates with increased cortical amyloid burden (Rebeck, G. W., *et al., Neuron. 11*:575-580 (1993)). *APOE* promoter polymorphisms, which upregulate transcription of *APOE*, have recently been shown to be associated with AD (Bullido, M. J., *et al., Nat. Genet. 18*:69-71 (1998); Lambert, J. C., *et al., Human Mol. Gen. 6*:533-540 (1998)). Higher expression of the *APOE-ε4* allele, relative to *APOE-ε3*, has been found in brains of *APOE-ε4* positive AD patients, but not in age- and genotype-matched controls (Lambert, J. C., *et al., Human Mol. Gen. 6*:2151-2154 (1997)). The absence of apoE in transgenic mice expressing FAD mutant APP attentuates Aβ deposition (Bales, K. R., *et al., Nature Genetics 17*:264 (1997)). The second gene linked to LOAD, the *LRP* gene, encodes the low density lipoprotein receptor-related protein. APP, apoE, and α_2M are all ligands for this cell-surface receptor

(Blacker, D. and Tanzi, R. E., *Archives of Neurology 55*:294-296 (1998); Kang, D. E., *et al., Neurology 49*:56-61 (1997); Blacker, D., *et al., Neurology 48*:139-147 (1997); Farrer, L. A., *et al., JAMA 278*:1349-1356 (1997); Strittmatter, W. J., *et al., Proc. Natl. Acad. Sci. USA 90*:1977-1981 (1993)). LRP internalizes ligands via endocytosis, and targets them for lysosomal degradation (Borth, W., *FASEB J. 6*:3345-3353 (1992)). Inheritance of a pentanucleotide deletion in the third gene associated with LOAD, *A2M* (*i.e*, inheritance of *A2M-2*), confers increased risk for AD and is present in ~30% of the population (Blacker, D., *et al., Nat. Genet. 19*:357-360 (1998)). The protein product of *A2M*, α_2M, is an abundant pan-protease inhibitor found primarily in serum, but is also present in brain and other organs (for example, liver). α_2M binds Aβ and can mediate its internalization and degradation (Borth, W., *FASEB J. 6*:3345-3353 (1992); Narita, M., *et al., J. Neurochem. 69*:1904-1911 (1997)).

α_2M has been implicated in the pathogenesis of AD by both biological and genetic findings. α_2M-like immunoreactivity was observed in AD cortical senile plaques (Bauer, J., *et al., FEBS Lett. 285*:111-114 (1991)) and it was shown that α_2M is upregulated in the AD brain where it localizes to neuritic but not diffuse amyloid plaques (Strauss, S., *et al., Lab. Invest 66*:223-230 (1992); Van Gool, D., *et al., Neurobiol. Aging 14*:233-237 (1993)). In addition, Aβ was found to bind to α_2M with high affinity (Du, Y., *et al., J. Neurochem. 69*:299-305 (1997)), and binding prevented amyloid fibril formation as well as neurotoxicity associated with aggregated Aβ (Du, Y., *et al., J. Neurochem. 70*:1182-1188 (1998); Hughes, S. R., *et al., Proc. Natl. Acad. Sci. USA 95*:3275-3280 (1998)). Activated α_2M-Aβ complexes were recently shown to be internalized and targeted for degradation by glioblastoma cells via binding to LRP (Narita, M., *et al., J. Neurochem. 69*:1904-1911 (1997)). Moreover, LRP is especially abundant in brain regions affected by AD such as the hippocampus (Rebeck, G.W., *et al., Neuron 11*:575-580 (1993); Tooyama, I., *et al., Mol. Chem. Neuropathol. 18*:153-160 (1993)), and serves as a receptor for ApoE (Rebeck, G.W., *et al., Neuron 11*:575-580 (1993)), a well established genetic risk factor (Blacker, D., *et al., Nature Gen. 19*:357-360 (1998)).

The genetic linkage of *APP, APOE, A2M,* and their receptor *LRP* to AD suggests that these proteins may participate in a common neuropathogenic pathway leading to AD (Blacker, D., *et al., Nat. Genet. 19*:357-360 (1998)). This pathway may be the α_2M mediated clearance and degradation of Aβ through α_2M binding to LRP for endocytosis and lysosomal degradation, and by serving as a direct mediator for Aβ degradation when α_2M is complexed with an unidentified serine protease (Qiu, W. Q., *et al., J. Biol. Chem. 271*:8443-8451 (1996)). This hypothesis is supported, *inter alia*, by the fact that apoE and α_2M are both ligands for *LRP* and, in addition, that apoE has previously been reported to inhibit α_2M mediated degradation of Aβ (Rebeck, G. W., *et al., Ann. Neurol. 37*:211-217 (1995); Zhang, Z., *et al., Int. J. Exp. Clin. Invest. 3*:156-161 (1996)).

However, in its normal role, α_2M also binds a host of cytokines, growth factors, and biologically active peptides (Borth, W., *FASEB J. 6*:3345-3353 (1992)). It has also recently been shown to activate the phosphatidylinositol 3-kinase suggesting a role in signaling (Misra, U. K. and Pizzo, S. V., *J. Biol. Chem. 273*:13399-13402 (1998)). Thus, defective activity of α_2M may lead to AD-related neurodegeneration by a variety of mechanisms beyond possible effects on Aβ accumulation and deposition.

A reduced steady-state level of secreted α_2M or the presence of defective tetramers due to dominant negative effects of *A2M-2* could result in impaired α_2M function. Partial or total deletion of the sequences coding for the bait region in exons 17 and 18 are likely to modify protease binding, activation, and internalization of potentially defective tetramers containing mutant monomer(s). Therefore, the generation of very low levels of mutant monomers may have an amplified effect as one mutant monomer may potentially inhibit the function of three wild-type monomers in the tetramer (dominant negative effect). Thus a critical role for α_2M is indicated in AD neuropathogenesis. The data described in Example 1 show that the *A2M-2* deletion leads to deleted/truncated forms of α_2M RNA and protein that may have a dominant negative effect on normal α_2M. Based on the finding, described herein, that the *A2M-2* deletion leads to the production of altered α_2M transcripts and proteins, strategies aimed at replacing or

supplementing normal α_2M function and activities, and/or at suppressing defective α_2M function in the brain may effectively serve as a means for therapeutically preventing, treating, or even reversing AD neuropathogenesis. In addition, these strategies may be useful for treating other pathologies associated with defective α_2M function. Moreover, methods based on the results and experiments described herein may be used to screen for these therapeutic agents.

The first aspect of present invention relates to therapeutic agents for AD that can replace or supplement normal α_2M function, and/or suppress expression of *A2M-2*.

In one embodiment of the invention, the therapeutic agent is an anti-LRP-Aβ molecule, which is a molecule containing LRP and Aβ binding domains. This molecule may be a peptide, or other molecule, that is capable of binding to both Aβ and LRP. This anti-LRP-Aβ molecule may also contain other domains. An anti-LRP-Aβ molecule having Aβ and LRP binding domains could bind Aβ and target it for LRP mediated endocytosis followed by lysosomal degradation, and thus would be useful, *inter alia*, as a therapeutic agent.

In one embodiment of the invention, the anti-LRP-Aβ molecule is a peptide, referred to herein as the anti-LRP-Aβ peptide. A 250-residue fragment of the α_2M monomer contains both the Aβ and LRP binding domains (Hughes, S. R., *et al., Proc. Natl. Acad. Sci. U.S.A. 95*:3275-3280 (1998)). Thus, in one embodiment of the invention, the anti-LRP-Aβ peptide would be composed of the entire Aβ and LRP binding domains of α_2M (SEQ ID NO:4). Alternatively, the Aβ and LRP binding domains may be composed of portions of the Aβ and LRP binding domains of α_2M. The Aβ binding domain of α_2M is located between residues 1201 and 1313, approximately 600 residues C-terminal to the bait region (Hughes, S.R., *et al., Proc. Natl. Acad. Sci. USA 95*:3275-3280 (1998)). Thus, in another embodiment of the invention, the Aβ binding domain of the anti-LRP-Aβ peptide would consist of the full Aβ binding domain of α_2M (between residues 1201-1313, SEQ ID NO:6), but only a portion of the LRP binding domain. In another embodiment of the invention, the Aβ binding domain would consist of at least 50 contiguous residues of the full Aβ binding domain of α_2M. In another

embodiment of the invention, the Aβ binding domain would consist of 10-50 contiguous residues of the full Aβ binding domain of α_2M.

In addition, peptides that can bind Aβ in vivo and inhibit Aβ fibril formation have been described by Soto *et al.*(Soto, C. *et al., Nat. Med. 4:*822-826 (1998); Soto, C., *et al., Biochem. Biophys. Res. Comm. 226:*672-680 (1996)). These peptides (SEQ ID NOs:12, 16, 18, 20, 22, 24 and 26) have homology to Aβ and a similar degree of hydrophobicity, but have a low propensity to adopt a β-sheet conformation. In particular one 11 residue Aβ binding peptide, having the amino acid sequence of SEQ ID NO:12, and encoded by the nucleic acid sequence of SEQ ID NO:11, was particularly effective. Therefore, in a preferred embodiment of the invention, the Aβ domain of the anti-LRP-Aβ peptide would have the sequence of this 11-residue peptide. Thus, in a preferred embodiment of the invention, the Aβ domain of the anti-LRP-Aβ peptide has the amino acid sequence of SEQ ID NO:12, and is encoded by the nucleic acid sequence of SEQ ID NO:11. Two shorter derivatives of this 11 residue Aβ binding peptide, composed of a 5 residue peptide (SEQ ID NO:22) and a 7 residue peptide (SEQ ID NO:18) also effectively bound Aβ and inhibited fibril formation (Soto, C. *et al., Nat. Med. 4:*822-826 (1998); Soto, C., *et al., Biochem. Biophys. Res. Comm. 226:*672-680 (1996)). Thus, in another preferred embodiment of the invention, the Aβ binding domain has the amino acid sequence of SEQ ID NO:22, and is encoded by the nucleic acid sequence of SEQ ID NO:21, or has the amino acid sequence of SEQ ID NO:18, and is encoded by the nucleic acid sequence of SEQ ID NO:17. Alternatively, the Aβ binding domain may be composed of other derivatives of the 11 residue Aβ binding peptide having 3, 4 or 6 residues (SEQ ID NO:24, 22 and 18 respectively). Thus in another embodiment of the invention, the Aβ binding domain has the amino acid sequence of SEQ ID NO:24, 22 or 18, and is encoded by the nucleic acid sequence of SEQ ID NO:23, 21 or 17, respectively.

The LRP binding domain of α_2M is located between residues 1312 and 1451 of α_2M, directly C-terminal to the Aβ binding domain (Hughes, S. R., *et al., Proc. Natl. Acad. Sci. USA 95:*3275-3280 (1998)). Thus, in one embodiment of

the invention, the LRP binding domain of the anti-LRP-Aβ peptide is composed of the full LRP binding domain of α_2M (residues 1313-1451, SEQ ID NO:8). In another embodiment of the invention, the LRP binding domain is composed of at least at least 50 contiguous residues of the full LRP binding domain of α_2M. In yet another embodiment of the invention, the LRP binding domain is composed of 10-50 contiguous residues of the full LRP binding domain of α_2M. Within the LRP binding domain, a 27 residue LRP binding consensus sequence exists at residues 1366-1392 (Nielsen, K. L., *et al., J. Biol. Chem. 271*:12909-12912 (1996)). Thus, in a preferred embodiment of the invention, the LRP binding domain of the anti-LRP-Aβ peptide is composed of residues 1366-1392 (SEQ ID NO:10) of α_2M. Alternatively, the LRP binding domain may be composed of a contiguous portion of residues 1313-1451 of α_2M that includes residues 1366-1392. In another preferred embodiment, the anti-LRP-Aβ peptide is composed of the 11 residue Aβ binding domain and the 27 residue consensus sequence of the α_2M LRP binding domain (SEQ ID NO:14).

The Aβ binding domain and the LRP binding domain of the anti-LRP-Aβ molecule may be connected to each other directly by a covalent bond, or indirectly by another molecule, such as a linker, or linkerless polyethylene glycol. Linker molecules include polymers such as polyethylene glycol (PEG) and peptides or amino acid residues. In addition, linkerless PEG modification (PEGylation) may be used (Francis, G. E., *et al., Int. J. Hematol. 68:*1-18 (1998)). Various methods of connecting molecules using linkers and other molecules are well known in the art, and may be used to connect the Aβ and LRP binding domains (See, for example, Francis, G. E., *et al., Int. J. Hematol. 68:*1-18 (1998); Raag, R. and Whitlow, M., *FASEB J. 9:*73-80 (1995); Deguchi, Y., *et al., Bioconjug. Chem. 10:*32-37 (1999); Luo, D., *et al., J. Biotechnol. 65:*225-228 (1998); Reiter, Y., and Pastan, I., *Clin Cancer Res. 2:*245-52 (1996); DeNardo, G. L., *et al., Clin. Canc. Res. 4:*2483-90 (1998); Taremi, S. S., *Protein Sci. 7:*2143-2149 (1998); Schaffer, D. V., and Lauffenburger, D. A., *J. Biol. Chem. 273:*28004-28009 (1998); Skordalakes, E., *et al., Biochem. 37:*14420-14427 (1998); Czerwinski, G., *et al., Proc. Natl. Acad. Sci. U.S.A. 95:*11520-11525 (1998); Daffix, L., *et al.,*

*J. Pept. Res. 52:*1-14 (1998); Liu, S. J., *et al., Blood 92:*2103-2112 (1998); Chandler, L. A., *et al., Int. J. Cancer 78:*106-111 (1998); Park, C. J., *Appl. Microbiol. Biotechnol. 50:*71-76 (1998); Suzuki, Y., *et al., J. Biomed. Mater. Res. 42:*112-116 (1998); Filikov, A. V., and James, T. L., *J. Comput. Aided Mol. Des. 12:*229-240 (1998); MacKenzie, R., and To, R., *J. Immunol. Methods 220:*39-49 (1998)).

In one preferred embodiment of the invention, the linker is composed of amino acid residues, for example, glycine residues or proline residues. Where the linker is composed of amino acid residues, it may be from 1-20 residues, but will preferably be 5-10 residues, and more preferably will be 5 residues.

Where the anti-LRP-Aβ molecule is a peptide, within the peptide, the Aβ binding domain may be C-terminal, or N-terminal to the LRP binding domain. However, preferably, the Aβ binding domain will be N-terminal to the LRP binding domain, which is the order of the Aβ and LRP binding domains in naturally occurring α_2M.

In addition, the invention provides for nucleic acid molecules that encode an anti-LRP-Aβ peptide. Thus, in another embodiment of the invention, the nucleic acid molecules would encode an anti-LRP-Aβ peptide having the sequences described above. The invention also relates to nucleic acids having at least 95% homology to these nucleic acids. In addition, the invention relates to nucleic acids that hybridize to a nucleic acid that is complementary to a nucleic acid encoding the anti-LRP-Aβ peptide. The conditions under which the first and second polynucleotides hybridize are preferably as follows: (a) incubate overnight at 42°C in a solution consisting of 50% formamide, 5x SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and a 20 μg/ml denatured, sheared salmon sperm DNA; and (b) wash at 65°C in a solution consisting of 0.1x SSC.

The anti-LRP-Aβ peptide may be produced using standard solid phase synthesis methods for protein synthesis, and purified by high performance liquid chromatography (HPLC) which are well known in the art (See "Preparation and Handling of Peptides," in: *Current Protocols in Protein Science*, Coligan, J. E.,

et al., eds., John Wiley and Sons, Inc., pub., Vol. 2., Chapter 18 (Suppl. 14 1998)). Alternatively, the anti-LRP-Aβ peptide may be produced using standard recombinant DNA methods. For example, The DNA coding for the desired sequence of the LRP binding domain (for example, the 27 residue consensus sequence) may be obtained by PCR amplification of the codons encoding the desired LRP binding domain using primers designed to flank the desired codons. This DNA may then be used as a template for PCR mediated integration of the sequence coding for the desired Aβ binding domain. For PCR mediated insertion of the Aβ domain, a nucleotide 5' PCR primer can be designed having (1) a region homologous to the end of the DNA sequence encoding the desired LRP binding domain that was amplified as described immediately above, and (2) immediately 5' to this region, a region encoding the desired Aβ binding domain, and (3) immediately 5' to this region a start codon. For the 3' primer, the 3' flanking primer used to amplify the LRP binding domain, which sequence is now being used as the template, may be used. Alternatively, to produce an anti-LRP-Aβ peptide having the entire Aβ and LRP binding domains of α_2M (residues 1202-1451), primers may be designed to flank the coding sequence for these domains, to amplify this region (nucleotides 3713-4465). A start codon may be then added by PCR mediated insertion. To amplify a coding region that encodes less than the entire AB and LRP binding domains, the primers may instead be designed to flank this smaller region of α_2M. The resulting nucleic acid molecule is DNA encoding a fusion protein having LRP and Aβ binding domains, and a start codon, such that this molecule may be inserted into an expression vector to produce the anti-LRP-Aβ peptide.

Once DNA encoding the desired fusion protein is obtained, PCR mediated insertion may be used to insert restriction enzyme sites at the 5' and 3' ends of the fusion gene so that the fusion protein gene may then be cleaved with these restriction enzymes for insertion into an expression vector, and a vector for use in the yeast three hybrid system (Tirode, F., *et al.*, *J.Biol. Chem. 272*:22995-22999 (1997)). For example, an Xho I and Kpn I restriction sites can be inserted at the 5' and 3' ends of the fusion protein gene, respectively. Cleavage with these

restriction enzymes will then facilitate cloning of the fusion protein gene into (i) the pBAD/His expression vector (Invitrogen), for arabinose dependent expression of anti-LRP-Aβ in *E. coli*, and (ii) the pLex9-3H vector for use in the yeast three hybrid system (Tirode, F., *et al., J.Biol. Chem. 272*:22995-22999 (1997)). The protein product, named anti-LRP-Aβ peptide, of the resulting gene should have both Aβ and LRP binding properties.

The ability of anti-LRP-Aβ molecule to bind Aβ and LRP and to undergo LRP mediated endocytosis and degradation may be tested using gel-filtration chromatography, immunoblotting and cell culture techniques. If the anti-LRP-Aβ molecule is a peptide, a yeast-three-hybrid system may also be used to evaluate the anti-LRP-Aβ peptide (Tirode, F., *et al., J. Biol. Chem. 272*:22995-22999 (1997)). If necessary, the binding properties of an anti-LRP-Aβ peptide may be reoptimized using *in vivo* evolution techniques (Buchholz, F., *et al., Nat. Biotechnol. 16*:657-662 (1998)).

Gel-filtration chromatograpy can be performed as described by Narita *et al.* (Narita, M., *et al., J. Neurochem. 69*:1904-1911 (1997)) to test the ability of an anti-LRP-Aβ molecule to bind Aβ. The anti-LRP-Aβ molecule is incubated with Aβ1-42 that is radiolabeled with ^{3}H, ^{14}C or ^{125}I. In the following discussion, ^{125}I-Aβ is used as an example of radiolabeled Aβ. Methylamine or trypsin activated α_2M, and α_2M, and unactivated α_2M and α_2M-2, may be used as controls. anti-LRP-Aβ/^{125}I-Aβ, α_2M/^{125}I-Aβ and α_2M-2/^{125}I-Aβ complexes are then separated from unbound ^{125}I-Aβ using a Superose 6 gel-filtration column (0.7 x 20 cm) under the control of an FPLC (Pharmacia) that has been standardized with molecular weight markers from 1000 kD- 4 kD. If anti-LRP-Aβ has bound ^{125}I-Aβ, ^{125}I-Aβ should be detected by gamma counter at two peaks, one corresponding to the molecular weight of the anti-LRP-Aβ/^{125}I-Aβ complex (about 8-9 kD for a complex containing an anti-LRP-Aβ of about 40 residues), and one corresponding to the molecular weight of ^{125}I-Aβ (4.5 kD).

Alternatively, or in addition to gel-filtration chromatography, immunoblotting methods (Narita, M., *et al., J. Neurochem. 69*:1904-1911 (1997)) may be used to determine whether an anti-LRP-Aβ molecule can bind Aβ.

Unlabeled Aβ is incubated separately with anti-LRP-Aβ, unactivated α_2M, unactivated α_2M-2, α_2M activated by methylamine or trypsin, or α_2M-2 activated by methylamine or trypsin. Samples are then electrophoresed on a 5% SDS-PAGE, under non-reducing conditions, transferred to polyvinyl difluoride nitrocellulose membrane, and probed with anti-Aβ IgG, or an antibody specific for the anti-LRP-Aβ molecule. Where one or more domains of the anti-LRP-Aβ molecule are derived from α_2M, an anti-α_2M IgG that recognizes the domain derived from α_2M may be used, such as anti-α_2M IgG raised against the LRP binding domain of α_2M (for example, Marynen, P., *et al., J. Immunol. 127*:1782-1787 (1981)). If the anti-LRP-Aβ/Aβ sample may be detected by both the antibody against anti-LRP-Aβ, and anti-Aβ IgG it can be concluded that the anti-LRP-Aβ molecule can bind Aβ. Where the Aβ binding domain of the anti-LRP-Aβ molecule is derived from Aβ, the anti-Aβ antibody should be tested to ensure that it does not recognize the anti-LRP-Aβ molecule. Several antibodies against Aβ are available, including 6310, WO2, 4G8, G210 and G211. Antibody 4G8 may recognize an anti-LRP-Aβ molecule for which Aβ binding domain is derived from Aβ. In addition, some anti-α_2M antibodies may not recognize an anti-LRP-Aβ molecule derived from α_2M, therefore, they should be tested for the ability to recognize the peptide prior to performing the immunoblotting, endocytosis, and degradation protocols described herein. Marynen *et al.*, (Marynen, P., *et al., J. Immunol. 127*:1782-1787 (1981)) describe an anti-α_2M antibody raised against the LRP binding domain that may be able to recognize an anti-LRP-Aβ peptide having an LRP binding domain derived from α_2M. Other anti-α_2M antibodies are available from Sigma and Cortex Biochem (San Leandro, CA, U.S.A.). α_2M can be obtained from Sigma, or purified from human plasma and activated as described in Warshawsky, I., *et al., J. Clin. Invest. 92*:937-944 (1993). Synthetic $A\beta_{1-42}$ can be purchased from Bachem (Torrance, CA, U.S.A.).

Gel-filtration chromatography and immunoblotting as described above may also be used to determine the ability of anti-LRP-Aβ to bind LRP, by using labeled soluble LRP (for example, the extracellular region of LRP) in place of labeled Aβ for gel-filtration chromatography experiments, and anti-LRP IgG in place of anti-

Aβ IgG for immunoblotting experiments. Alternatively, for the immunoblotting protocol, the anti-LRP-Aβ molecule may be labeled with fluorescent or radioactive label. For a labeled anti-LRP-Aβ molecule, it can be concluded that the anti-LRP-Aβ molecule can bind Aβ if the labeled band corresponds to a band recognized by anti-Aβ antibody.

The ability of Aβ/anti-LRP-Aβ complexes to undergo LRP mediated endocytosis and subsequent degradation can be determined using cell culture experiments using cells that express LRP as described by Kounnas *et al.* (Kounnas, M. Z., *et al., Cell 82:*331-340 (1995); Kounnas, M. Z., *et al., J. Biol. Chem. 270:*9307-9312 (1995)). The amount of radioligand that is internalized or degraded by cells has been described previously (Kounnas, M. Z., *et al., Cell 82:*331-340 (1995); Kounnas, M. Z., *et al., J. Biol. Chem. 270:*9307-9312 (1995)). Cells that express LRP include, but are not limited to, adipocytes, astrocytes, fibroblasts, hepatocytes, macrophages, monocytes, and syncytiotrophoblasts. In one preferred embodiment of the invention, mouse embryo fibroblasts are used for the cell culture experiment.

Cells expressing LRP are incubated for 18 hours with ^{125}I-Aβ (alternatively, Aβ may be labeled with ^{3}H or ^{14}C) in the presence or absence of anti-LRP-Aβ, unactivated α_2M, unactivated α_2M-2, α_2M activated by methylamine or trypsin, or α_2M-2 activated by methylamine or trypsin; in the presence or absence of RAP (400 nM). RAP is an inhibitor of LRP ligand binding, and is added to determine if endocytosis is LRP mediated. RAP can be isolated and purified from a glutathione *S*-transferase fusion protein expressed in *E. coli* as described in Warshawsky, I., *et al., J. Clin. Invest. 92:*937-944 (1993b). To assess endocytosis rather than degradation, chloroquine (0.1 mM) is added at the same time as anti-LRP-Aβ/^{125}I-Aβ to inhibit lysosomal degradation of ^{125}I-Aβ.

The amount of radioactive ligand released by treatment with trypsin-EDTA, proteinase K solution defines the surface-bound material, and the amount of radioactivity associated with the cell pellet defines the amount or internalized ligand. Activated α_2M/^{125}I-Aβ will serve as positive control. Under the conditions described, more than 8 fmoles / 10^4 cells of activated α_2M/^{125}I-Aβ should be

internalized after 18 hours of incubation (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995)). Unactivated α_2M/^{125}I-Aβ will serve as the negative control for endocytosis, because α_2M must be activated by trypsin or methylamine to be recognized by LRP. If the amount of anti-LRP-Aβ/^{125}I-Aβ is greater than 4-8 fmoles/10^4 cells, it can be concluded that anti-LRP-Aβ/^{125}I-Aβ has the ability to undergo LRP mediated endocytosis. Unactivated α_2M/^{125}I-Aβ, and activated α_2M/^{125}I-Aβ in the presence of RAP should not be internalized, therefore no more than 2-4 fmoles/10^4 cells should be internalized (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995)). Internalization of the anti-LRP-Aβ/^{125}I-Aβ complex will be deemed abolished if anti-LRP-Aβ/^{125}I-Aβ, in the presence and absence of RAP, and unactivated α_2M/ ^{125}I-Aβ show the same amount of radioactivity associated with the cell pellet.

To determine the ability of Aβ/anti-LRP-Aβ complexes to undergo degradation after endocytosis, this cell culture protocol is repeated without chloroquine. The radioactivity appearing in the cell culture medium that is soluble in 10% trichloroacetic acid is taken to represent degraded ^{125}I-Aβ (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995); Narita, M., *et al.*, *J. Neurochem. 69*:1904-1911 (1997)). Total ligand degradation is corrected for the amount of degradation that occurs in control wells lacking cells. Because free ^{125}I-Aβ can be degraded in an LRP independent manner, degradation is measured for anti-LRP-Aβ and α_2M complexes with ^{125}I-Aβ, as well as for free ^{125}I-Aβ, in the presence and absence of RAP. Using the same positive and negative controls as above, if RAP does not decrease the amount of TCA soluble radioactivity by at least 30% for the anti-LRP-Aβ/^{125}I-Aβ complex, it can be concluded that ^{125}I-Aβ ligand of anti-LRP-Aβ is not degraded.

Another method of testing the ability of anti-LRP-Aβ molecule to bind Aβ and LRP is the yeast three-hybrid system described by Tirode *et al.* (Tirode, F., *et al.*, *J. Biol. Chem. 272*:22995-22999 (1997)). This method may be used where the anti-LRP-Aβ molecule is a peptide. In this system, yeast growth only occurs when the "bait" recognizes both the "hook" and the "fish" (Figure 7). In this instance, the "hook" is constructed of the DNA coding for Aβ (Bales, K. R., *et al.*,

Nat. Genet. 17:264 (1997)), fused to the coding sequence of the LexA DNA binding protein in pLex9-3H, a *TRP1* episomal vector (Tirode, F., *et al., J. Biol. Chem. 272*:22995-22999 (1997)). The "fish" is constructed of the coding sequence for the 515kD extracellular domain of LRP, fused to the B42 activation domain in pVP 16, a *LEU2* episomal vector (Tirode, F., *et al., J. Biol. Chem. 272*:22995-22999 (1997)). The "bait" is the DNA coding for anti-LRP-Aβ in the pLex9-3H vector, expression of anti-LRP-Aβ is repressed by methionine. After transformation of yeast with these vectors, transcription of the Leu 2 reporter gene will occur only when the Aβ fused DNA binding domain is brought into proximity to the transcriptional activation domain fused to LRP. The Aβ/LRP binding fusion peptide should promote reporter gene transcription. The interaction between anti-LRP-Aβ and Aβ and LRP (515 kD) will be considered positive only if reporter gene expression (yeast growth) occurs when Aβ-LexA, LRP(515kD)-B42, and anti-LRP-Aβ are expressed.

The anti-LRP-Aβ molecule of the invention may be administered per se, or in the form of a pharmaceutically acceptable salt with any non-toxic, organic or inorganic acid. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acid, and acid metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids which form suitable salts include the mono, di and tricarboxylic acids. Illustrative of such acids are, for example, acetic, glycolic, lactic, pyruvic, malonic, succinic, glutaric, fumaric, malic, tartaric, citric, ascorbic, maleic, hydroxymaleic, benzoic, hydroxybenzoic, phenylacetic, cinnamic, salicylic, 2-phenoxybenzoic and sulfonic acids such as methane sulfonic acid and 2-hydroxyethane sulfonic acid. Salts of the carboxy terminal amino acid moiety include the non-toxic carboxylic acid salts formed with any suitable inorganic or organic bases. Illustratively, these salts include those of alkali metals, as for example, sodium and potassium; alkaline earth metals, such as calcium and magnesium; light metals of Group IIIA including aluminum; and organic primary, secondary and tertiary amines, as for example, trialkylamines, including triethylamine, procaine, dibenzylamine, 1-ethenamine,

N,N'-dibenzylethylenediamine, dihydroabietylamine, N-(lower)alkylpiperidine, and any other suitable amine.

The amount of the anti-LRP-Aβ molecule administered to a subject will vary depending upon the age, weight, and condition of the subject. The course of treatment may last from several days to several months or until a cure is effected or a diminution of disease state is achieved, or alternatively may continue for a period of years, for example, when used prophylactically. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. However, the amount of anti-LRP-Aβ molecule administered to a subject is generally from 0.1 nanograms to 10 mg/kg/day, and is typically an amount ranging from 1 nanogram to 1 mg/kg/day.

The present invention also relates to antisense oligonucleotides targeted to *A2M-2* RNA, and to their use as therapeutic agents for AD and for suppressing *A2M-2* expression. Partial or total deletion of the sequences coding for the bait region in exons 17 and 18 of α_2M is likely to modify protease binding, interfering with α_2M activation. Incorporation of one or more mutant monomers into tetramers might thereby result in defective tetramers that could not be activated efficiently and, therefore, could not undergo subsequent endocytosis via LRP. Thus, the generation of very low levels of mutant monomers may have an amplified effect as one mutant monomer may potentially inhibit the function of three wild-type monomers in the tetramer (dominant negative effect). One way to counter this dominant negative effect is to decrease the level of abnormal α_2M by interfering with gene expression at the RNA level. For this purpose, an antisense oligonucleotide specific for *A2M-2* RNA can be used. This oligonucleotide will be referred to herein as *A2M-2* antisense oligonucleotide. The *A2M-2* antisense oligonucleotide may be targeted to any *A2M-2* RNA molecule, but in a preferred embodiment of the invention, it is targeted to heterologous nuclear (hnRNA).

The *A2M-2* deletion is found in the splicing region of exon 18, therefore, in one embodiment of the invention, the *A2M-2* antisense oligonucleotide is

designed to target *A2M-2* RNA transcripts before splicing occurs, referred to as hnRNA. In addition, in order to be specific for *A2M-2* hnRNA the *A2M-2* antisense oligonucleotide is designed to target the pentanucleotide deletion found in *A2M-2*. In another embodiment of the invention, the *A2M-2* antisense oligonucleotide is designed to target *A2M-2* mRNA. The *A2M-2* deletion results in several variant mRNA transcripts with varying sequences. The *A2M-2* antisense oligonucleotides of the invention can be designed to target individual variants, or to target more than one of these variants. In addition, *A2M-2* antisense oligonucleotides targeting different *A2M-2* mRNA variants, or targeting *A2M-2* hnRNA, may be used either alone, or in conjunction with one another.

In addition, the *A2M-2* antisense oligonucleotide must be long enough so that it targets only *A2M-2*, but short enough to optimize delivery. Thus, the antisense oligonucleotide of the invention is preferably 8-50 nucleotides in length, and more preferably 15-30 nucleotides in length. Therefore, in one embodiment of the invention, the *A2M-2* antisense oligonucleotide is 8-50 nucleotides and is complementary to the coding strand of the region of *A2M-2* containing the site of the pentanucleotide deletion. In a preferred embodiment of the invention, the *A2M-2* antisense oligonucleotide is composed of 15-30 contiguous nucleotides of a region complementary to the site on the coding strand of *A2M-2* that contains the pentanucleotide deletion. In another embodiment of the invention, the *A2M-2* antisense oligonucleotide is composed of the last 8-50 contiguous nucleotides of SEQ ID NO:27. In a preferred embodiment of the invention, the *A2M-2* antisense oligonucleotide is composed of the last 15-30 contiguous nucleotides of SEQ ID NO:27. In yet another preferred embodiment, the *A2M-2* antisense oligonucleotide is composed of nucleotides 36-50 of SEQ ID NO:27. In another preferred embodiment of the invention, the *A2M-2* antisense oligonucleotide is composed of nucleotides 20-50 of SEQ ID NO:27.

The *A2M-2* antisense oligonucleotide may be DNA or RNA, *i.e.*, it may be composed of deoxyribonucleic acids or ribonucleic acids, respectively. Alternatively, the oligonucleotide may be composed of nucleotides with a phosphorothioate backbone to render the oligonucleotide more resistant to

enzymatic degradation (van der Krol, A. R., *et al.*, *Biotechniques 6:*958-976 (1988); Cazenave, C. & & Hélène, C., "Antisense Oligonucleotides," in: *Antisense nucleic acids and proteins: Fundamental and applications*, Mol, J. N. M. & van der Krol, A. R., eds., M. Dekker, publ., New York, pp. 1-6 (1991); Milligan, J. F., *et al., J. Med. Chem. 36:*1923-1937 (1993)). In a preferred embodiment of the invention the *A2M-2* antisense oligonucleotide is DNA.

Other modifications which may be used to protect the oligonucleotide include chemical changes to the 3' end of the oligonucleotide (van der Krol, A. R., *et al., Biotechniques 6:*958-976 (1988); Khan, I. M. & Coulson, J. M., *Nucleic Acids Res. 21:*2957-2958 (1993); Tang, J. Y., *et al. Nucleic Acids Re. 21:*2729-2735 (1993)) or biotynylation of the 3' end followed by conjugation with avidin (Boado, R. J. & Pardridge, W. M., *Bioconjugate Chem. 3:*519-523 (1992)). Alternatively, lipofection may be used to deliver the oligonucleotide, *i.e.*, packaging the oligonucleotide in lipid (McCarthy, M. M., *et al., Endocrin. 133:*433-439 (1993b); Ogawa, S., *et al., J. Neurosci. 14:*1766-1774 (1994)). Lipofection protects the oligonucleotide from nucleases and may aid in delivery to the central nervous system.

The *A2M-2* antisense oligonucleotide can be easily synthesized by means of commercially-available automatic DNA synthesizers such as a DNA synthesizer manufactured by Applied Biosystems, or MilliGen, etc. In addition, methods of synthesizing oligonucleotides are well known in the art and are described, for example, in *Oligonucleotides and Analogues a Practical Approach*, Eckstein, F., ed.,Oxford University Press, publ. New York, (1991), and "Synthesis and Purification of Oligonucleotides" in: *Current Protocols in Molecular Biology*, Ausubel, F. M., *et al.*, eds., John Wiley & Sons, Inc., publ., Vol. 1, §§ 2.11-2.12 (Suppl. 9 1993).

The invention also relates to pharmaceutical compositions containing the *A2M-2* antisense oligonucleotide, and one or more pharmaceutically acceptable carriers. In addition, the invention provides a method of treating AD and/or of suppressing *A2M-2* expression by administering the *A2M-2* antisense oligonucleotide to a subject. Preferably, the *A2M-2* antisense oligonucleotide is

delivered to a subject who has been determined to be heterozygous or homozygous for the *A2M-2* allele. Procedures for selecting and assessing subjects who are heterozygous or homozygous for *A2M-2* are described in Tanzi *et al.*, U.S. Serial No. 09/148,503, PCT Application No. PCT/US98/18535, and Blacker, D., *et al.*, *Nat. Genet. 19*:357-360 (August 1998). In another preferred embodiment of the invention, treatment of a subject with the *A2M-2* antisense oligonucleotide is done in conjunction with a therapy designed to replace or supplement α_2M function.

Antisense oligonucleotides have been safely administered to humans and several clinical trials are presently underway. Based on these clinical trials, oligonucleotides are understood to have toxicities within acceptable limits at dosages required for therapeutic efficacy. One such antisense oligonucleotide, identified as ISIS 2105, is presently in clinical trials, and is used as a therapeutic against papillomavirus. Another antisense oligonucleotide, ISIS 2922, has been shown to have clinical efficacy against cytomegalovirus-associated retinitis *Antiviral Agents Bulletin 5:* 161-163 (1992); *BioWorld Today*, Dec. 20, 1993. Therefore, it has been established that oligonucleotides are useful therapeutic agents and that they can be used for treatment of animals, especially humans.

The amount of the *A2M-2* antisense oligonucleotide administered to a subject will vary depending upon the age, weight, and condition of the subject. The course of treatment may last from several days to several months or until a cure is effected or a diminution of disease state is achieved, or alternatively may continue for a period of years, for example, when used prophylactically. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}'s in *in vitro* and *in vivo* animal studies. In general, dosage is from 0.01 mg to 100 g and may be given once daily, weekly, monthly or yearly.

Another therapeutic method of the invention is gene therapy to supplement α_2M function. Because the *A2M-2* deletion may result in impaired α_2M function,

a strategy aimed at supplementing normal α_2M, such as gene therapy, could serve as a means for treating, preventing or reversing AD. One embodiment of the invention is a viral vector carrying a transgene encoding wild type α_2M, or an anti-LRP-Aβ peptide. Viral vectors suitable for use in the invention are those that are capable of transfecting nondividing, post-mitotic cells, and have low cytotoxicity. These vectors include, but are not limited to adenovirus, lentivirus, and HSV-1, but are preferably adeno-associated virus vector (AAV). AAV is a DNA virus that is not directly associated with any human disease, and therefore should present a lower risk of cytotoxicity (Freese, A. *et al.*, *Epilesia 38:*759-766 (1997)). It can transfect nondividing, post-mitotic cells, such as neurons and dormant glial cells. In addition, there is some evidence that AAV may stably integrate into the host chromosome (Freese, Z. *et al.*, *Mov. Disord. 11:*469-488 (1996); Kaplitt, M. G. *et al.*, *Natur. Genet. 8:*148-154 (1994); Samulski, R. J., *et al.*, *J. Virol 63:*3822-3888 (1989); Kotin, R. M. *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87:*2211-2215 (1990); Samulski, R. J. *et al.*, *E.M.B.O. J. 10:*3941-3950 (1991); Muzyczka, N., *Curr. Topics. Microbiol. Immunol. 158:* 97-129 (1992)). Recently, AAV was successfully used to deliver a reporter transgene to human hippocampal tissue (Freese, A. *et al.*, *Epilesia 38:*759-766 (1997)).

Transgenes to be used in the viral vector include the full length cDNA encoding α_2M (SEQ ID NO:1), or the anti-LRP-Aβ peptide described above. The construction of AAV*lacZ* is described by Kaplitt, *et al.*, and Samulski *et al.* (Kaplitt, M. G., *et al.*, *Nature Genet. 8:*148-154 (1994); Samulski, R. J., *et al.*, *J. Virol. 63:*3822-3888 (1989)). To insert the transgene into the viral vector, the viral vector is first cut with restriction enzymes. PCR mediated integration is used to create corresponding restriction sites at the 3' and 5' ends of the transgene, and the transgene is ligated with AAV.

The invention also provides a method of combating AD by administering the viral vector carrying an α_2M, or an anti-LRP-Aβ peptide transgene and pharmaceutical compositions containing this viral vector.

The gene therapy of the invention can be administered using *in vivo* or *ex vivo* strategies. The *in vivo* approach involves the introduction of the viral vector

directly into the tissue of the subject. *In vivo* methods of administration include direct injection into cerebrospinal fluid, or by stereotactic intracerebral inoculation into the hippocampus. In addition, some viral vectors, such as adenovirus, can be transported in a retrograde manner from the point of injection (Ridoux, V., *et al., Brain Res. 648:*171-175 (1994); Kuo, H., *et al., Brain Res. 24:*31-38 (1995)). Other routes of administration include nasal inhalation (Draghia, R., *Gene Ther. 2:*418-423 (1995)) and injection into the carotid artery after disruption of the blood brain barrier (Doran, S. E., *et al., Neurosurgery 36:*965-970 (1995); Muldoon, L. L., *Am. J. Pathol. 147:*1840-1851 (1995)).

For the *ex vivo* approach, a suitable cell type, such as fibroblasts myoblasts, or neural progenitor cells, is harvested from a donor and grown in tissue culture. The cells are then transfected, and the cells harvested and implanted in the subject. *Ex vivo* methods are described, for example,at Raymon, H. K., *et al., Exper. Neurol. 144:*82-91 (1997); Rosenberg, M. B., *et al., Science 2442:*1575-1578 (1988); Suhr, S. T., and Gage, F. H., *Arch. Neurol. 50:*1252-1268 (1993); Tuszynski, M. H., *et al.,Exp. Neurol. 126:*1-14 (1994); Ridoux, V. *et al., Neuroreport 5:*801-804 (1994); Buc-Caron, M. H., *Neurobiol. Dis 2:*37-47 (1995); Sabaté, O., *et al., Nat. Genet. 9:*256-260 (1995).

The amount of viral vector carrying a transgene administered to a subject will vary depending upon the age, weight, and condition of the subject. The course of treatment may last from several days to several months or until a cure is effected or a diminution of disease state is achieved, or alternatively may continue for a period of years, for example, when used prophylactically. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. In general, dosage is from 1×10^4 to 1×10^{10} plaque forming units (pfu), but is preferably 1×10^6 to 5×10^7 pfu/kg and may be given once daily, weekly, monthly or yearly.

The therapeutic agents of the invention can be administered alone, or in concert with one another or with other therapeutic agents. For example, a subject may be treated with both the anti-LRP-Aβ molecule and the antisense

oligonucleotide of the invention, to provide both a supplement of *A2M* function, and to block defective *A2M* function at the same time.

Suitable subjects for carrying out the present invention are typically male or female human subjects, and include both those which have previously been determined to be at risk of developing AD, and those who have been initially diagnosed with AD. The present invention may be employed in combating both familial AD (late onset and early onset) as well as sporadic AD. One preferable group of subjects are those who have been determined to be heterozygous or homozygous for the *A2M-2* allele. Procedures for selecting and assessing subjects who are heterozygous or homozygous for *A2M-2* are described in Tanzi *et al.*, U.S. Serial No. 09/148,503, PCT Application No. PCT/US98/18535, and Blacker, D., *et al.*, *Nat. Genet. 19*:357-360 (August 1998), all of which are herein incorporated by reference.

When the therapeutic agents as mentioned above are used as preventive or therapeutic agents for Alzheimer's disease, they may be made into preparations which satisfy the necessary requirements of the particular administering route together with usual carriers. For example, in the case of oral administration, preparations in the form of tablets, capsules, granules, diluted powder, liquid, etc. are prepared.

Pharmaceutical compositions containing the therapeutic agents of the invention, may be prepared in either solid or liquid form. To prepare the pharmaceutical compositions of this invention, one or more of the therapeutic agents is intimately admixed with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques, which carrier may take a wide variety of forms depending on the form of preparation desired for administration, for example, oral or parenteral. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material, or formulation auxiliary of any type. In preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed. Thus, for liquid oral preparations, such as for example, suspensions, elixirs and solutions, suitable carriers and additives include water, glycols, oils, alcohols, flavoring

agents, preservatives, coloring agents and the like; for solid oral preparations such as, for example, powders, capsules and tablets, suitable carriers and additives include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. In addition to such pharmaceutical carriers, cationic lipids may be included in the formulation to facilitate oligonucleotide uptake. One such composition shown to facilitate uptake is LIPOFECTIN (GIBCO-BRL, Bethesda, Md.).

Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are employed. If desired, tablets may be sugar coated or enteric coated by standard techniques. For parenterally injectable compositions, the carrier will usually comprise sterile, pyrogen-free water, or sterile, pyrogen-free physiological saline solution, though other ingredients, for example, for purposes such as aiding solubility or for preservatives, may be included. Parenterally injectable suspensions (for example, for intravenous or intrathecal injection) may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed. See generally *Remington's Pharmaceutical Sciences* (18th ed.) Mack Publishing Co. (1990).

The pharmaceutical compositions of this invention may be administered in a number of ways depending upon whether local or systemic treatment is desired, and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral, for example, by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection or intrathecal or intraventricular administration. Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable. Compositions for intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

When necessary, the pharmaceutical composition may be prepared so that the therapeutic agent passes through the blood-brain barrier. One way to accomplish transport across the blood-brain barrier is to couple or conjugate the therapeutic agent to a secondary molecule (a "carrier"), which is either a peptide or a non-proteinaceous moiety. The carrier is selected such that it is able to penetrate the blood-brain barrier. Examples of suitable carriers are pyridinium, fatty acids, inositol, cholesterol, and glucose derivatives. Alternatively, the carrier can be a compound which enters the brain through a specific transport system in brain endothelial cells, such as transport systems for transferring insulin, or insulin-like growth factors I and II. This combination of therapeutic agent and carrier is called a prodrug. Upon entering the central nervous system, the prodrug may remain intact or the chemical linkage between the carrier and therapeutic agent may be hydrolyzed, thereby separating the carrier from the therapeutic agent. See generally U.S. Pat. No. 5,017,566 to Bodor.

An alternative method for transporting the therapeutic agent across the blood-brain barrier is to encapsulate the carrier in a lipid vesicle such as a microcrystal or liposome. Such lipid vesicles may be single or multi-layered, and encapsulate the therapeutic agent either in the center thereof or between the layers thereof. Such preparations are well known. For example, PCT Application WO 91/04014 of Collins et al. describes a liposome delivery system in which the therapeutic agent is encapsulated within the liposome, and the outside layer of the liposome has added to it molecules that normally are transported across the blood-brain barrier. Such liposomes can target endogenous brain transport systems that transport specific ligands across the blood-brain barrier, including but not limited to, transferring insulin, and insulin-like growth factors I and II. Alternatively, antibodies to brain endothelial cell receptors for such ligands can be added to the outer liposome layer. U.S. Pat. No. 4,704,355 to Bernstein describes methods for coupling antibodies to liposomes.

Another method of formulating the therapeutic agent to pass through the blood-brain barrier is to prepare a pharmaceutical composition as described above, wherein the therapeutic agent is encapsulated in cyclodextrin. Any suitable cyclodextrin which passes through the blood-brain barrier may be employed, including β-cyclodextrin, γ-cyclodextrin, and derivatives thereof. See generally U.S. Pat. No. 5,017,566 to Bodor; U.S. Pat. No. 5,002,935 to Bodor; U.S. Pat. No. 4,983,586 to Bodor.

Another method of passing the therapeutic agent through the blood-brain barrier is to prepare and administer a pharmaceutical composition as described above, with the composition further including a glycerol derivative as described in U.S. Pat. No. 5,153,179 to Eibl.

An alternative method of delivering the therapeutic agent to the brain is to implant a polymeric device containing the agent, which device is able to provide controlled release delivery of the agent to the brain for an extended period after implantation. Examples of such implantable polymeric devices are described in U.S. Pat. No. 5,601,835 to Sabel, and in U.S. Pat. No. 5,846,565, to Brem.

Another aspect of the invention relates to methods of screening for therapeutic agents for AD that can replace or supplement normal α_2M function and activities, and/or suppress defective α_2M function.

The invention provides for a method of screening for therapeutic agents for AD that can suppress the production of RNA encoding α_2M-2 variants, and thereby suppress the production of α_2M-2 variants. One embodiment of the invention is a method for screening for therapeutic agents by incubating cells that are heterozygous or homozygous for *A2M-2*, and that express *A2M-2*, with a test agent, and determining whether the agent increases the ratio of normal to aberrant *A2M* mRNA. Preferably the cells used are heterozygous for the *A2M-2* allele, with the other allele being *A2M-1* (*A2M*-1/2 cells). Examples of cells that may be used for this assay include, but are not limited to, glioma cells, hepatocytes, and hepatoma cell lines. In addition, cells used for the assay may be transformed or transfected to enable them to grow indefinitely in culture. To screen for these agents, the cells carrying are incubated with the test agent, preferably, for a period

ranging from 2 hours to 24 hours. The incubation period may be longer or shorter depending on the agent, and suitable incubation periods can be determined by one of ordinary skill in the art. Cells homozygous for *A2M-1* are used as a control. Procedures for *A2M-2* genotyping are described in Tanzi *et al.*, U.S. Serial No. 09/148,503, PCT Application No. PCT/US98/18535, and Blacker, D., *et al.*, *Nat. Genet. 19*:357-360 (August 1998). After incubation, the ratio of normal to aberrant α_2M mRNA transcripts is determined, and compared to the ratio for cells (with the same genotype as the cells treated with agent) untreated with agent, and for *A2M*-1/1 cells untreated with agent. An increase in the ratio of normal to aberrant α_2M mRNA transcripts relative to this ratio for cells untreated with the agent would indicate an effective agent. This ratio for *A2M*-1/2 cells untreated with an agent is typically from 5:1 to 20:1. If the ratio of normal to aberrant α_2M mRNA transcripts approaches the ratio found in *A2M*-1/1 cells untreated with agent, the agent will be considered effective. Thus, for example, if the ratio in *A2M*-1/2 cells is 10:1, and the ratio in *A2M*-1/1 cells is100:1, a test agent that results in the ratio to 20:1 would be considered effective.

The ratio of normal to aberrant transcripts may be quantitated by S1 nuclease analysis, or by RT PCR on RNA isolated from the glioma cells. Protocols for RNA isolation for cells in culture, and for S1 nuclease analysis is described in "Preparation and Analysis of RNA" in: *Current Protocols in Molecular Biology*, Ausubel, F. M., *et al.*, eds., John Wiley & Sons, Inc., publ., Vol. 1, § 4 (Suppl. 37 1997). S1 nuclease analysis is performed using a single-stranded antisense probe encompassing at least exons 17-18 (bp 2057-2284 of SEQ ID NO:1), synthesized from a full length *A2M* cDNA template (SEQ ID NO:1). Preferably, the probe would encompass exons 17, 18 and part of exon 19. The length of the probe is preferably from 250 bp to 500 bp long, and is more preferably 300 bp long. The probe may be up to 4353 bp (the length of the coding region), however, increasing the length of the probe may decrease the accuracy of the assay. In a preferred embodiment of the invention, the probe is complementary to nucleotides 2024-2323 of SEQ ID NO:1, in another preferred embodiment, the probe is complementary to nucleotides 2057-2384 of SEQ ID

NO:1. After the RNA has been hybridized with the probe, and digested with S1 nuclease, samples are run on a polyacrylamide gel with molecular weight markers. Wild type mRNA transcript (*A2M-1*) should appear as a band corresponding to the length of the probe, for example, 300 bp, *A2M-2* variant transcripts should appear as smaller bands. Total normal mRNA to total variant mRNA is compared and the ratio of normal to aberrant determined.

Alternatively, RT PCR may be used to quantitate mRNA transcripts. Protocols for RT PCR are described in "The Polymerase Chain Reaction" in: *Current Protocols in Molecular Biology*, Ausubel, F. M., *et al.*, eds., John Wiley & Sons, Inc., publ., Vol. 2, § 15.4 (Suppl. 17 1992). RNA isolated from the treated and control cells is amplified using RT PCR with labeled primers designed to amplify a region including at least exons 17-18 (bp 2057-2284 of SEQ ID NO:1), and preferably exons 17, 18 and part of exon 19. In addition, the primers may designed to target mRNA by synthesizing them so that they bind to the junction of two exons. For example, in a preferred pair of primers, the first primer would hybridize to *A2M* cDNA encoding the end of exon 16 and beginning of exon 17, and the second primer would hybridize to *A2M* cDNA encoding the end of exon 18, and beginning of exon 19. The primers may be from 8-50 nucleotides in length, but are preferably 15-30 nucleotides in length, and are more preferably 15 nucleotides in length. The PCR product is then run on a polyacrylamide gel with molecular weight markers. Bands corresponding to wild type mRNA transcripts should correspond to the length of *A2M-1* cDNA corresponding to the far ends of the primers used. For example, wild type mRNA amplified by primers designed to amplify the last 5 base pairs of exon 16 to the first 5 base pairs of exon 19 (bp 2052-2289 of SEQ ID NO:1), would be 238 nucleotides. If the primers were designed to amplify a region starting at the beginning of exon 17, including exon 18, and ending after the first 100 nucleotides of exon 19 (bp 2057-2456 of SEQ ID NO:1) the expected fragment length would be 400 nucleotides for normal mRNA. Variant mRNA transcripts will be shorter. Total normal mRNA to total variant mRNA is compared and the ratio of normal to aberrant determined.

Other methods of RNA quantitation that may be used in the invention are well known in the art, and are described in, *for example*, *PCR Protocols, A Guide to Methods and Applications*, Innis, A., *et al.*, eds., Academic Press, Inc., San Diego, CA, pub., pp. 60-75 (1990).

Another embodiment of the invention is to screen for nontoxic agents that can activate α_2M through mechanisms other than cleavage of the bait domain. For α_2M tetramers having one or more α_2M-2 monomers, protease activation of the bait domain may be impaired. Because activation is required to expose the LRP binding domain, impairment of activation of one or more monomers of a tetramer would result in a decreased ability to bind to LRP. Consequently, these tetramers would be inefficient at clearing Aβ through LRP mediated endocytosis. However, α_2M may be activated through mechanisms other than protease cleavage of the bait domain. For example, agents other than proteases, such as methylamine, activate α_2M through the thiolester site. These agents would be able to activate defective α_2M monomers, exposing the LRP binding domain (and other domains) and potentially allowing for LRP mediated clearance of Aβ. In addition, these agents could be used to increase the amount of active wild type α_2M tetramers, to compensate for defective α_2M tetramers. Presently, effective nontoxic agents capable of activating α_2M at sites other than the bait domain are unknown. The invention provides for a method of screening for such agents.

To screen for these agents, α_2M is treated with a test agent, and then tested to determine whether it has undergone a conformational change, or for its ability to bind to LRP. The α_2M used for the assay may be wild type α_2M, α_2M-2, or α_2M mutants that are missing all, or a portion of the bait domain. However, preferably, wild type α_2M is used. In addition, α_2M used for the assay may be in the form of dimers or tetramers, but is preferably in the form of tetramers. For treatment of α_2M with the test agent, the α_2M is preferably incubated with the test agent for 2-24 hours. However, the incubation period may be longer or shorter according to the agent, and suitable incubation periods can be determined by one of ordinary skill in the art. To determine whether treated α_2M has undergone a conformational change, the α_2M electrophoretic-mobility assay may be used. To

determine the ability of treated α_2M to bind to LRP, any method of measuring LRP binding may be used, however, preferred methods include enzyme-linked immunosorbent assays (ELISA), immunoblotting, LRP mediated endocytosis, and LRP mediated degradation.

The α_2M electrophoretic mobility assay can also be used to determine whether treated α_2M has been activated, by determining whether treated α_2M has undergone the conformational change expected for activated α_2M. The α_2M electrophoretic-mobility assay consists of analyzing the electrophoretic mobility of α_2M under non-denaturing conditions after incubation with the test agent, or as a control, a protease, or other reagent capable of converting α_2M to the fast form (Barret, A. J., *et al.*, *Biochem. J. 181:* 401-418 (1979); Bowen, M. E., and Gettins, P. W., *J. Biol. Chem. 273:*1825-1831 (1998)). α_2M can exist in two forms easily distinguishable by mobility in gel electrophoresis (Barret, A. J., *et al.*, *Biochem. J. 181:* 401-418 (1979)). The difference in mobility is due to the conformational change that α_2M undergoes after activation with a protease or other agent, such as methylamine. This conformational change results in an increase in electrophoretic mobility on poly-acrylamide gels run under non-denaturing conditions (this form is referred to as the "fast form" of α_2M) (Barret, A. J., *et al.*, *Biochem. J. 181:* 401-418 (1979); Bowen, M. E., and Gettins, P. W., *J. Biol. Chem. 273:*1825-1831 (1998)). This "slow to fast" conversion is used as the basis for an assay for this conformational change, and the two different α_2M conformations are referred to as the slow and fast forms (Bowen, M. E., and Gettins, P. W., *J. Biol. Chem. 273:*1825-1831 (1998)). Conversion from the slow to fast form for α_2M treated with a test agent would indicate that the agent had activated α_2M. Where this assay is used to determine the effectiveness of a test agent, the α_2M treated with the agent would preferably be tetrameric.

The α_2M electrophoretic mobility assay and methods of purifying α_2M from serum are described by Barret *et al.* in Barret, A. J., *et al.*, *Biochem. J. 181:* 401-418 (1979), and by Bowen *et al.* in Bowen, M. E., *et al.*, *Arch. Biochem. Biophys. 337:*191-201 (1997), and in Bowen, M. E., and Gettins, P. W., *J. Biol. Chem. 273:*1825-1831 (1998). After incubation with the test agent, the α_2M

sample may be run on polyacrylamide gel under nondenaturing conditions, such as those described in Bowen, M. E., *et al.*, *Arch. Biochem. Biophys. 337:*191-201 (1997). The α_2M sample may be detected by methods well known in the art such as by radiolabelling the protease used, or by Western Blot using anti-α_2M antibodies. Activated and unactivated α_2M may be used as controls for comparison of electrophoretic mobility with the sample being analyzed.

In one embodiment of the invention, ELISA is used to determine the ability of treated α_2M to bind to LRP. ELISA protocols are described in "Immunology" in: *Current Protocols in Molecular Biology*, Ausubel, F. M., *et al.*, eds., John Wiley & Sons, Inc., publ., Vol. 2, § 11.2 (Suppl. 15 1991). In this assay, microtiter plate wells coated with an anti-α_2M IgG that recognizes only activated α_2M, such as the antibody described by Marynen *et al.*, (Marynen, P., *et al.*, *J. Immunol. 127:* 1782-1786 (1981)), are incubated with the treated α_2M, or control molecule. The wells are then incubated with an enzyme-conjugated anti-α_2M IgG and rinsed. Next, the wells are incubated with the substrate for the enzyme conjugate, rinsed, and the amount of α_2M sample bound in the well is determined. Alternatively, microtiter plate wells are coated with anti-LRP IgG and rinsed. The wells are then incubated with LRP and rinsed. This LRP is preferably soluble LRP. Then the wells are incubated with α_2M treated with the test agent, untreated α_2M, or activated α_2M, and rinsed. Next the wells are incubated with enzyme-conjugated anti-α_2M IgG, rinsed again, and then incubated with the substrate for the enzyme that is conjugated to the anti-α_2M IgG. The amount of α_2M sample bound in the well is then determined. In another embodiment, wells coated with LRP are incubated with α_2M treated with the test agent, untreated unactivated α_2M, or untreated activated α_2M, and rinsed. The wells are then incubated with enzyme-conjugated anti-α_2M IgG, rinsed, and then treated with the enzyme substrate, and the amount of α_2M sample bound is determined. The anti-α_2M IgG may be conjugated with, for example, horseradish peroxidase, urease or alkaline phosphatase, but is preferably labeled with a fluorescent label, such as 4-methylumbelliferyl phosphate (MUP). The appropriate substrate is added to the wells, the wells are washed, and then quantitated with a microtitre plate reader.

Alternatively, the ability of α_2M treated with the test agent to bind to LRP may be determined by immunoblotting methods. Unlabeled soluble LRP is incubated separately with α_2M treated with the test agent, untreated unactivated α_2M, and untreated α_2M activated by methylamine or trypsin. Samples are then electrophoresed on a 5% SDS-PAGE, under non-reducing conditions, transferred to polyvinyl difluoride nitrocellulose membrane, and probed with anti-α_2M IgG and anti-LRP IgG. If the α_2M treated with the test agent may be detected by both anti-α_2M IgG and anti-LRP IgG it can be concluded that the treated α_2M can bind Aβ. In another method of immunoblotting, an antibody specific for the LRP binding domain of α_2M, such as that described by Marynen, *et al.*, (Marynen, P., *et al., J. Immunol. 127:* 1782-1786 (1981)), is used as the anti-α_2M IgG, and the samples are not incubated with LRP. Recognition of the treated α_2M by this antibody indicates that α_2M has been activated.

In addition, the ability of α_2M treated with a test agent to bind to LRP can be determined by its ability to undergo LRP mediated endocytosis using cell culture experiments as described by Kounnas *et al.* (Kounnas, M. Z., *et al., Cell 82:*331-340 (1995); Kounnas, M. Z., *et al., J. Biol. Chem. 270:*9307-9312 (1995)). Cells expressing LRP, mouse embryo fibroblasts, are incubated for 18 hours with ^{125}I-Aβ (alternatively, Aβ may be labeled with ^{3}H or ^{14}C) in the presence or absence of with α_2M treated with the test agent, untreated unactivated α_2M, and untreated α_2M activated by methylamine or trypsin, in the presence or absence of RAP (400 nM). RAP is an inhibitor of LRP ligand binding, and is added to determine if endocytosis is LRP mediated. In addition, chloroquine (0.1 mM) is added to inhibit lysosomal degradation of ^{125}I-Aβ.

The amount of radioactive ligand released by treatment with trypsin-EDTA, proteinase K solution defines the surface-bound material, and the amount of radioactivity associated with the cell pellet defines the amount of internalized ligand. Activated α_2M/^{125}I-Aβ will serve as positive control. Under the conditions described, more than 4-8 fmoles/10^4 cells of activated α_2M/^{125}I-Aβ should be internalized after 18 hours of incubation (Kounnas, M. Z., *et al., Cell 82:*331-340 (1995)). Unactivated α_2M/^{125}I-Aβ and activated α_2M/^{125}I-Aβ in the presence of

RAP should not be internalized, therefore, no more than 2-4 fmoles/10^4 cells should be internalized. If the amount of test agent treated α_2M/^{125}I-Aβ is greater than 4-8 fmoles/10^4 cells, it can be concluded that α_2M/^{125}I-Aβ has the ability to undergo LRP mediated endocytosis. In addition, unactivated α_2M/^{125}I-Aβ, and activated α_2M/^{125}I-Aβ in the presence of RAP should not be internalized, therefore no more than 2-4 fmoles/10^4 cells should be internalized (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995)). Internalization of the treated α_2M/^{125}I-Aβ complex will be deemed abolished if treated α_2M/^{125}I-Aβ, in the presence and absence of RAP, and unactivated α_2M/ ^{125}I-Aβ show the same amount of radioactivity associated with the cell pellet.

To determine the ability of treated α_2M/Aβ complexes to undergo degradation after endocytosis, this cell culture protocol is repeated without chloroquine. The radioactivity appearing in the cell culture medium that is soluble in 10% trichloroacetic acid is taken to represent degraded ^{125}I-Aβ (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995); Narita, M., *et al.*, *J. Neurochem. 69*:1904-1911 (1997)). Total ligand degradation is corrected for the amount of degradation that occurs in control wells lacking cells. Because free ^{125}I-Aβ can be degraded in an LRP independent manner, degradation is measured for treated α_2M, and untreated α_2M complexes with ^{125}I-Aβ, as well as for free ^{125}I-Aβ, in the presence and absence of RAP. Using the same positive and negative controls as above, if RAP does not decrease the amount of TCA soluble radioactivity by at least 30% for the treated α_2M/^{125}I-Aβ complex, it can be concluded that ^{125}I-Aβ ligand of treated α_2M is not degraded.

It will be readily apparent to those skilled in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein are obvious and may be made without departing from the scope of the invention or any embodiment thereof. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

Example 1

In view of the link between the inheritance of *A2M-2*, and the role of α_2M in brain, the potential effects of the *A2M-2* deletion polymorphism on *A2M* mRNA and on the α_2M protein were investigated. These studies were complicated by the fact the polymorphism does not directly alter the coding sequence of α_2M, but consists of an intronic deletion just before the exon 18 splice acceptor site (Matthijs, G., *et al.*, *Nucleic Acids Res. 19*:5102 (1991)). If exon 18 were to be deleted as a result of the *A2M-2* polymorphism, this deletion would result in the loss of half of the active center or "bait" region of α_2M (specifically, deletion of the last 20 amino acids out of the 39 amino acids forming the bait region), with likely adverse functional consequences for α_2M activity. With specific regard to Aβ, the peptide does not directly bind to the bait region. However, recognition and cleavage of the bait domain by target proteases is a necessary prerequisite *in vivo* for activation of α_2M via a conformational change in the α_2M tetramer. Activation of α_2M then results in the presentation of the LRP-binding domains which is essential for binding to LRP (Borth, W., *FASEB J. 6*:3345-3353 (1992)). Thus, clearance of α_2M ligands (for example, cytokines, growth factors, Aβ), would be hampered by deletion of the bait domain (exon 18).

A specific deletion of exon 18 due to the *A2M-2* deletion would also result in a frame-shift in the coding region in exon 19, resulting in the synthesis of a truncated α_2M monomer. Therefore, one likely consequence of a modification of the bait region is the formation of a defective α_2M tetramer (insertion of defective monomer) which could not be activated and undergo subsequent endocytosis via LRP. Experiments with an exon 18 deleted α_2M construct expressed in cells indicate that a truncated α_2M protein at the bait region can still be secreted and form tetramers with itself. In addition, only human glioma cell lines positive for the *A2M-2* allele produced altered *A2M* message and corresponding truncated α_2M monomers consistent with a deletion of exon 18 followed by termination of the amino acid sequence in exon 19.

Methods and Results

First, the effect of the *A2M-2* deletion on RNA splicing and on α_2M complex formation and secretion were investigated. To study the biological effects of the *A2M-2* polymorphism in an endogenous system, 15 human glioblastoma cell lines expressing high levels of α_2M were genotyped (Blacker, D., *et al., Nature Genetics 19*:357-360 (1998)). While the highest levels of α_2M would be expected from hepatoma cell lines, glioblastomas were chosen because of their CNS origin. Ten primary glioblastoma cell lines (all derived from different patients) were homozygotes for the *A2M-1* (no deletion) allele, while 3 cell lines were *A2M*-1/2 heterozygotes for the deletion. Two cell lines did not qualify for either of these alleles and were excluded from further studies. At the molecular level, the *A2M-2* allele consists of a deletion of 5 bp (ACCAT) in the consensus polypyrimidine tract immediately prior to the consensus 3' AG at the splice acceptor site of exon 18 (Matthijs, G., *et al., Nucleic Acids Res. 19*:5102 (1991)). Given the position of the polymorphism, aberrant *A2M* RNA splicing might be expected to lead to a deletion at exon 18 since the consensus polypyrimidine tract would be reduced by 3 pyrimidines (to a minimal consensus configuration for exon splicing). Deletion of exon 18 would, in turn, result in termination of the protein due to a stop codon in exon 19. Reverse transcription-PCR (RT-PCR) was employed in attempts to identify aberrant splice products in the vicinity of exon 18 of the *A2M* gene. An expected 399 bp fragment encompassing exons 17, 18, and 19 was amplified by RT-PCR of RNA isolated from the 13 human glioma cell lines. Agarose gel/ethidium bromide staining was not sensitive enough to reveal aberrant *A2M* transcripts in any of the cell lines containing the *A2M-2* allele. However, using polyacrylamide gels, various ^{33}P-labeled PCR products ranging in size between 250-290 bp were detected. These products were found exclusively in the *A2M*-1/2 cell lines (Figure 1).

Next, these products were cloned into the vector pCR 2.1. Four different clones representing aberrant mRNA transcripts have been identified using this approach (Figure 2). Sequencing of these clones revealed aberrant splicing events around exon 18 leading to the production of variably sized RNAs in which exon

17 and/or 19 may also be shortened. Clone 1 has a 208 bp deletion (2126-2334) including exon 18 and, interestingly, also 42 and 50 bp of exons 17 and 19, respectively. The protein product resulting from such a deletion would still be in frame with 69 amino acids missing, including most of the bait region. Clones 2, 3, and 4 contain unidentified DNA fragments which continue within exon 19 to bp 2355, 2320, and 2297 respectively. The unknown sequences are most likely intronic sequences that are not accessible in DNA databases. Therefore, aberrant splicing events around exon 18 do not appear to simply result in the precise deletion of exon 18. Rather, they lead to the production of variably sized RNAs in which exons 17 and/or 19 may also be partly deleted.

Next, experiments designed to detect mutant forms of α_2M protein containing large deletions or truncations were performed. Based on the low level of aberrant mRNA transcripts, the expected amount of mutant proteins could be below detection or not recognized by the antibody used, since the antibody was raised against the holoprotein. Finally, a truncated or grossly altered protein may be targeted by the quality control system in the ER for degradation prior to secretion. These concerns were addressed by producing an *A2M* cDNA construct in which a stop codon is inserted in the middle of exon 18 and transfecting this construct into chinese hamster ovary (CHO) cells, which do not produce α_2M endogenously. As seen in Figure 3, both media and extracts from the transfected cells contained truncated and the control full-length α_2M protein products. The gels shown were run under denaturing but non-reducing conditions. Under these conditions, monomers of the truncated protein and monomers and dimers of the full-length protein were detected in the cell lysate. In the media, however, almost all of the truncated protein formed tetramers, and dimers were barely detectable. Wild-type full-length α_2M was also present in the media mainly in the form of tetramers and dimers. Besides demonstrating that the antibody used is able to recognize the N-terminal half of α_2M and that a truncated α_2M protein can be synthesized and secreted by CHO cells, the results of this experiment (Figure 3) also provided preliminary data indicating that secreted α_2M levels may dramatically decrease as a result of the truncation.

Next, the effects of the *A2M*-2 polymorphism on secretion and tetramer formation of endogenous α_2M were examined. For this purpose, endogenous secreted α_2M was analyzed by Western blot analysis. Glioblastoma cells were cultured overnight in OptiMem (Gibco) serum-free media (as bovine serum contains high levels of α_2M), and secreted α_2M was immunoprecipitated with a polyclonal α_2M antibody obtained from Sigma. When the immunoprecipitate was resolved by SDS PAGE, the expected 180 kD monomer was detected in all lines tested, however, smaller aberrant forms of α_2M were detected only in the *A2M-2* positive cells. Figure 4 shows cell lysates from wild-type and *A2M-2* deletion-bearing cells. The data revealed protein bands consistent with truncated forms of α_2M exclusively in the *A2M-2* deletion-containing cells. The media (data not shown) from *A2M-1* and *A2M-2* cells contained primarily full-length α_2M monomers, but in the media from the *A2M-2* cells small amounts of truncated species could also be observed .

Discussion

A reduced steady-state level of secreted α_2M, or the presence of defective tetramers due to dominant negative effects of *A2M-2*, could result in impaired α_2M function. Partial or total deletion of the sequences coding for the bait region in exons 17 and 18 are likely to modify protease binding, activation, and internalization of potentially defective tetramers containing mutant monomer(s). Therefore, the generation of very low levels of mutant monomers may have an amplified effect as one mutant monomer may potentially inhibit the function of three wild-type monomers in the tetramer (dominant negative effect). Based on these and the linkage between the *A2M-2* deletion and AD (Blacker, D., *et al.*, *Nat. Genet. 19*:357-360 (1998)), a critical role for α_2M is indicated in AD neuropathogenesis. The data described herein show that the *A2M-2* deletion leads to deleted/truncated forms of α_2M RNA and protein that may have a dominant negative effect on normal α_2M.

Example 2

To test the *A2M-2* antisense oligonucleotides of the invention, and the S1 nuclease assay, A2M-2 antisense oligonucleotides having the nucleotide sequences of nucleotides 35-50, and 20-50 of SEQ ID NO:27 are synthesized using an automatic DNA synthesizer (MilliGen). The oligonucleotides recovered from 20% acrylamide-urea gel, and purified by means of an ethanol precipitating method, and the precipitate is dissolved in water at a concentration of 1 μmol. *A2M-2* sense oligonucleotides complementary to each of the antisense nucleotides are used as a positive control. Each of the antisense or sense oligonucleotides (1 μmol) is added to 1 ml cell culture medium. Each 1ml sample is then incubated with glioma cells heterozygous for the *A2M-2* allele, or homozygous for wild type *A2M* (*A2M-1*) at 37° C for 24 hours. The cells are washed with phosphate buffered saline, and homogenized in a denaturing solution containing 4 M guanidine thiocyanate. RNA is extracted using phenol/chloroform extraction and ultracentrifugation. The RNA pellet is then rinsed with 1ml 75% ethanol/25% 0.1 M sodium acetate, and resuspended in 100 μl water. RNA from each sample is then probed using a 300 bp antisense DNA probe encompassing exons 17 and 18 (nucleotides 2057-2356 of the full length cDNA for α^2M (SEQ ID NO:1)) end labeled with ^{32}P. The probe is hybrized with 15 μg RNA from each sample. The RNA is then precipitated, washed and resuspended with S1 hybridization solution. The samples are then denatured for 10 minutes at 65°C, and hybridized overnight at 30°C. 300 U S1 nuclease buffer in 150 μl S1 nuclease buffer with single-stranded calf thymus DNA is then added to each sample and incubated for 60 minutes at 30°C. The reaction is stopped, the RNA precipitated, washed, and resuspended, and the samples are run on a polyacrylamide gel with molecular weight markers. Wild type transcripts (*A2M-1*) should appear as 300 bp bands, *A2M-2* variant transcripts should appear as smaller bands. Without *A2M-2* antisense oligonucleotide treatment, this ratio is expected to be approximately 10:1 wild type to variant mRNA. The ratio of wild type to variant transcripts is

determined and compared to the ratio found for *A2M* mRNA from *A2M*-1/1 cells.

Example 3

To screen for therapeutic agents capable of activating α_2M through a site other than the bait domain, unactivated tetrameric α_2M (Sigma) (about 1mg/ml) is incubated with 5, 20, 50 or 100 μg of test agent in Tris/HCl or sodium phospate buffer at 37°C for 2 hours. Untreated unactivated α_2M, and untreated α_2M activated with methylamine or trypsin are used as controls.

Microtiter plates are incubated for 2h at 37° with 50 μl of LRP (10 μg)/well, and then rinsed with deionized water. The plates are then filled with blocking buffer and rinsed. 50 μl of treated α_2M, untreated unactivated α_2M, or untreated α_2M activated with methylamine or trypsin is added to each well and incubated for 2h at room temperature. After rinsing, 50 μl anti-α_2M IgG conjugated with MUP in blocking buffer is added to the wells and incubated for 2h at room temperature. After rinsing, MUP substrate is added to the wells, and incubated for 1 h at room temperature. The amount of α_2M bound is quantitated with a spectrofluorometer with a 365-nm excitation filter and 450 μm emission filter.

Example 4

Given the evidence that only a few key interactions are required for α_2M binding to LRP and Aβ (as discussed above), a small peptide containing LRP and Aβ binding domains could promote Aβ binding, LRP mediated endocytosis, and finally Aβ degradation. Such a peptide could serve as a substitute for α_2M-2 if it is not able to promote Aβ clearance and degradation.

Protein-protein interactions are usually mediated by a few key interactions (Wells, J. A., *Proc. Natl. Acad. Sci. U.S.A. 93*:1-6 (1996)). The Aβ clearance properties of α_2M do not require all the domains of an intact 5804 residue α_2M tetramer. A 250-residue fragment of the α_2M monomer contains both the Aβ and LRP binding domains (Hughes, S. R., *et al.*, *Proc.*

Natl. Acad. Sci. U.S.A. 95:3275-3280 (1998)). An 11-residue peptide can bind Aβ *in vivo* and a 27 residue LRP binding consensus sequence exists (Soto, C., *et al., Nat. Med. 4*:822-826 (1998); Nielsen, K. L., *et al., J. Biol. Chem. 271*:12909-12912 (1996); Soto, C., *et al., Biochem. Biophys. Res. Commun. 226*:672-680 (1996)). A peptide containing an Aβ and an LRP binding domain could bind Aβ and target it for LRP mediated endocytosis followed by lysosomal degradation. To achieve this goal, first, a peptide consisting of an 11-residue Aβ binding peptide and a 27 residue LRP binding domain is produced and tested for Aβ binding and clearance properties. If necessary, the binding properties of this anti-LRP-Aβ peptide can be reoptimized using *in vivo* evolution techniques (Buchholz, F., *et al., Nat. Biotechnol. 16*:657-662 (1998)).

Methods

Figure 6 shows the sequence of one possible anti-LRP-Aβ peptide. Using standard solid phase synthesis methods this peptide is synthesized in quantities sufficient to carry out tests to determine function in Aβ clearance. (*See* "Preparation and Handling of Peptides," in: *Current Protocols in Protein Science*, Coligan, J. E., *et al.*, eds., John Wiley and Sons, Inc., pub., Vol. 2., Chapter 18 (Suppl. 14 1998)). DNA encoding the fusion peptide is then synthesized. The DNA coding for the 27 residue LRP binding peptide is obtained by PCR amplification of codons 1366 to 1392 of the *A2M* gene (Nielsen, K. L., *et al., J. Biol. Chem. 271*:12909-12912 (1996)). To integrate the 11 residue Aβ binding sequence into the LRP binding sequence PCR mediated insertion is used. A 55 nucleotide 5' PCR primer is designed that has 25 nucleotides of homology to the LRP binding sequence and 36 nucleotides corresponding to the 11 residues of the Aβ binding peptide and a start codon. PCR mediated insertion is also used to insert an Xho I and Kpn I restriction enzyme sites at the 5' and 3' ends of the fusion gene, respectively. Cleavage with these enzymes will facilitate cloning of the fusion protein gene into (i) the pBAD/His expression vector (Invitrogen), for arabinose dependent expression

of anti-LRP-Aβ in *E. coli*, and (ii) the pLex9-3H vector for use in the yeast three hybrid system (Tirode, F., *et al.*, *J.Biol. Chem. 272*:22995-22999 (1997)). The protein product, named anti-LRP-Aβ, of the resulting gene should have both Aβ and LRP binding properties.

Aβ Binding. The ability of anti-LRP-Aβ to bind Aβ is first determined by gel-filtration chromatography and immunoblotting. Both of these methods have been used successfully by other investigators to investigate Aβ binding to wild type and variant α_2M (Narita, M., *et al.*, *J. Neurochem. 69*:1904-1911 (1997); Du, Y., *et al.*, *J. Neurochem. 69*:299-305 (1997)). Aβ1-42 is iodinated with ^{125}I, following the procedure of Narita *et al.* (Narita, M., *et al.*, *J. Neurochem. 69*:1904-1911 (1997)). ^{125}I-Aβ (5 nmol) is incubated separately with anti-LRP-Aβ, unactivated α_2M, unactivated α_2M-2, α_2M activated by methylamine or trypsin, or α_2M-2 activated by methylamine or trypsin. A ten fold molar excess of Aβ is used and the samples are incubated in 25 mM Tris-HCl, 150 mM NaCl, pH 7.4 for two hours at 37°C. Controls containing only ^{125}I-Aβ are also incubated. The anti-LRP-Aβ/^{125}I-Aβ, α_2M/^{125}I-Aβ, and α_2M-2/^{125}I-Aβ complexes are separated from unbound ^{125}I-Aβ using a Superose 6 gel-filtration column (0.7 X 20 cm) under the control of an FPLC (Pharmacia). 25 MM Tris-HCl, 150 mM NaCl, pH 7.4 are used to equilibrate the column and elute the samples. Using a flow rate of 0.05 ml/minute, 200 μL fractions are collected. Having standardized the column with molecular weight markers ranging from 1000 kD to 4 kD, anti-LRP-Aβ/^{125}I-Aβ, α_2M/^{125}I-Aβ, and α_2M-2/^{125}I-Aβ fractions are counted in a γ counter to determine the elution profile of ^{125}I-Aβ. If anti-LRP-Aβ has bound ^{125}I-Aβ, ^{125}I-Aβ should be detected by gamma counter at two peaks, one corresponding to the molecular weight of the anti-LRP-Aβ/^{125}I-Aβ complex (about 8-9 kD for this anti-LRP-Aβ peptide), and one corresponding to the molecular weight of ^{125}I-Aβ (4.5 kD).

It is unlikely, but possible, that iodinated Aβ may lead to a false positive or negative binding. Therefore, immunoblotting experiments are undertaken to confirm the results of the gel-filtration chromatography experiment (Narita, M., *et al.*, *J. Neurochem. 69*:1904-1911 (1997); Du, Y., *et al.*, *J. Neurochem.*

69:299-305 (1997)). Unlabeled Aβ is incubated separately with anti-LRP-Aβ, unactivated α_2M, unactivated α_2M-2, α_2M activated by methylamine or trypsin, or α_2M-2 activated by methylamine or trypsin, under the same conditions described above. Samples are electrophoresed on a 5% SDS-PAGE, under non-reducing conditions, and transferred to polyvinyl difluoride nitrocellulose membrane (Immobilon-P). These membranes are probed with polyclonal anti-α_2M IgG or monoclonal anti-Aβ IgG. Immunoreactive proteins are visualized using ECL and peroxidase conjugated anti-rabbit IgG. Molecular mass markers are used to determine if the immunoreactive proteins from the anti-α_2M and anti-Aβ blots for corresponding lanes display the same mobility. If the immunoreactive proteins display the same mobility then it will be concluded that Aβ binds anti-LRP-Aβ.

Endocytosis. The ability of anti-LRP-Aβ/Aβ complexes to undergo LRP mediated endocytosis and subsequent degradation is determined in cell culture experiments. The amount of radioligand that is internalized or degraded by cells has been described previously (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995); Kounnas, M. Z., *et al.*, *J. Biol. Chem. 270*:9307-9312 (1995)). Mouse embryo fibroblasts, which are cells that express LRP, are plated in 12 well plates to a density of 2 x 10^5 cells per well, and grown for 18 hours at 37°C in 5% CO_2. Cells are incubated in 1% Nutridoma (Boehringer Mannheim), penicillin/streptomycin, 1.5% bovine serum albumin for one hour prior to addition of ^{125}I-Aβ in the presence or absence of anti-LRP-Aβ, unactivated α_2M, unactivated α_2M-2, α_2M activated by methylamine or trypsin, or α_2M-2 activated by methylamine or trypsin, in the presence or absence of RAP (400 nM). To assess anti-LRP-Aβ/^{125}I-Aβ endocytosis by LRP, chloroquine (0.1 mM) is added at the same time as anti-LRP-Aβ/^{125}I-Aβ (4 nM) to inhibit lysosomal degradation of ^{125}I-Aβ (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995)).

Following 18 hours of incubation with the anti-LRP-Aβ/^{125}I-Aβ, cells are washed with phosphate-buffered saline and treated with a trypsin-EDTA, proteinase K solution. Surface-bound material is defined as the amount of

radioactive ligand released by this treatment, and the amount of internalized ligand is defined as the amount of radioactivity which remains associated with the cell pellet following the treatment.

Activated α_2M/ ^{125}I-Aβ will serve as positive control. Under the conditions described, more than 4-8 fmoles / 10^4 cells of activated α_2M/^{125}I-Aβ should be internalized after 18 hours of incubation (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995)). Unactivated α_2M/^{125}I-Aβ will serve as the negative control, because α_2M must be activated by trypsin or methylamine to be recognized by LRP. If the amount of anti-LRP-Aβ/^{125}I-Aβ is greater than 2-4 fmoles/10^4 cells, it can be concluded that anti-LRP-Aβ/^{125}I-Aβ has the ability to undergo LRP mediated endocytosis. Unactivated α_2M/^{125}I-Aβ, and activated α_2M/^{125}I-Aβ in the presence of RAP should not be internalized, therefore no more than 2-4 fmoles/10^4 cells should be internalized (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995)). Internalization of the anti-LRP-Aβ/^{125}I-Aβ complex will be deemed abolished if anti-LRP-Aβ/^{125}I-Aβ, in the presence and absence of RAP, and unactivated α_2M/ ^{125}I-Aβ show the same amount of radioactivity associated with the cell pellet.

Degradation. The experiment above to test endocytosis is repeated without chloroquine. The radioactivity appearing in the cell culture medium that is soluble in 10% trichloroacetic acid is taken to represent degraded ^{125}I-Aβ (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995); Narita, M., *et al.*, *J. Neurochem. 69*:1904-1911 (1997)). Total ligand degradation is corrected for the amount of degradation that occurs in control wells lacking cells. Because free ^{125}I-Aβ can be degraded in an LRP independent manner, degradation is measured for anti-LRP-Aβ and α_2M complexes with ^{125}I-Aβ as well as for free ^{125}I-Aβ in the presence and absence of RAP. Using the same positive and negative controls as above, if RAP does not decrease the amount of TCA soluble radioactivity by at least 30% for the anti-LRP-Aβ/^{125}I-Aβ complex it can be concluded that ^{125}I-Aβ ligand of anti-LRP-Aβ is not degraded.

The anti-LRP-Aβ peptide may not promote Aβ binding and degradation because of steric constrains. If the anti-LRP-Aβ polypeptide does not promote

Aβ binding and degradation another peptide is synthesized with a penta-glycine linker between the Aβ and LRP binding regions to provide the flexibility needed to bind both targets simultaneously. This anti-LRP-Aβ with linker is tested for Aβ binding, and LRP mediated endocytosis and degradation as described above. If this anti-LRP-Aβ does not provide for Aβ and LRP binding, the three hybrid system is used to reoptimize binding, and to screen for anti-LRP-Aβ with the ability to bind both Aβ and LRP.

The use of peptides in therapy is associated with two problems, transport across the blood-brain barrier, and the generation of an immune response. These problems can be minimized by shortening the peptide length. Thus when optimizing the anti-LRP-Aβ peptide, shorter binding domains may be preferred over longer domains, where binding capabilities are equally effective.

Yeast three hybrid system. The yeast three hybrid system is a genetic method to detect ternary protein complex formation (Figure 7) (Tirode, F., *et al., J. Biol. Chem. 272*:22995-22999 (1997); Osborne, M.A., *et al., Biotechnology 13*:1474-1478 (1995); Zhang, J. and Lautar, S., *Anal. Biochem. 242*:68-72 (1996); Licitra, E. J. and Liu, J. O., *Proc. Natl. Acad. Sci. U.S.A. 93*:12817-12821 (1996)). In the system, yeast growth only occurs when the "bait" recognizes both the "hook" and the "fish" (Figure 7). In this instance, the "hook" is constructed of the DNA coding for Aβ (Bales, K. R., *et al., Nat. Genet. 17*:264 (1997)), fused to the coding sequence of the LexA DNA binding protein in pLex9-3H, a *TRP1* episomal vector (Tirode, F., *et al., J. Biol. Chem. 272*:22995-22999 (1997)). The "fish" is constructed of the coding sequence for the 515kD extracellular domain of LRP, fused to the B42 activation domain in pVP 16, a *LEU2* episomal vector (Tirode, F., *et al., J. Biol. Chem. 272*:22995-22999 (1997)). The "bait" is the DNA coding for anti-LRP-Aβ in the pLex9-3H vector, expression of anti-LRP-Aβ is repressed by methionine. These vectors are transformed into the L40 yeast strain. Transcription of the Leu 2 reporter gene occurs only when the Aβ fused DNA binding domain is brought into proximity to the transcriptional activation domain fused to LRP.

The Aβ/LRP binding fusion peptide should promote reporter gene transcription. The interaction between anti-LRP-Aβ and Aβ and LRP (515 kD) will be considered positive only if reporter gene expression (yeast growth) occurs when Aβ-LexA, LRP(515kD)-B42, and anti-LRP-Aβ are expressed. It is not likely that expression of Aβ-LexA will cause activation of the reporter transcription since this construct has been used successfully in the past. It is also unlikely that LRP(515kD)-B42 expression alone will cause reporter transcription, LRP(515kD) is not known to bind DNA. The interaction of Aβ-LexA and LRP(515kD)-B42 would cause reporter transcription and the Aβ parent protein APP is known to interact with LRP. However, the interaction between LRP and APP occurs via the Kunitz protease inhibitory domain far removed from the location of Aβ in APP (Kounnas, M. Z., *et al.*, *Cell 82*:331-340 (1995)). In addition biochemical evidence suggests that LRP does not recognize Aβ (Narita, M., *et al.*, *J. Neurochem. 69*:1904-1911 (1997)). Transformation of the Aβ-LexA and LRP(515kD)-B42 containing plasmids into EGY48 and monitoring the growth on media lacking leucine is carried out to insure that Aβ-LexA and LRP(515kD)-B42 do not interact. As positive controls the DNA sequence encoding the entire α_2M monomer and the sequence encoding residues 1202-1451 of α_2M are cloned separately into pLex9-3H, in place of anti-LRP-Aβ. The C-terminal fragment of α_2M contains the full length Aβ and LRP binding domains (residues 1202-1451 of α_2M) and it, along with the monomer, should give rise to reporter gene transcription.

If expression of anti-LRP-Aβ, Aβ-LexA, and LRP(515kD)-B42 does not activate reporter transcription then each of the binary interactions of anti-LRP-Aβ are tested in a traditional two hybrid screen. That is, concomitant expression of anti-LRP-Aβ-B42 and Aβ-LexA, as well as anti-LRP-Aβ-B-42 and LRP(515kD)-LexA, is used to assess the ability of anti-LRP-Aβ to interact with Aβ-LexA and LRP(515kD)-LexA individually. If anti-LRP-Aβ interacts individually with both targets then one or all of the following is carried out: (i) a 5 residue glycine linker is added between the Aβ binding domain and the LRP binding to allow flexibility between the two binding domains, (ii) the Aβ-LexA

and LRP(515kD)-B42 fusion partners are switched to become LRP(515kD)-LexA and Aβ-B42, and (iii) the polarity of the anti-LRP-Aβ is switched so that the LRP binding domain is N-terminal to the Aβ binding domain. If anti-LRP-Aβ interacts with one or neither of the targets, binding is reoptimized using random mutagenesis and selection by three hybrid screen for binding to both targets. The non-binding region of anti-LRP-Aβ is subjected to protein evolution techniques, error prone PCR and DNA shuffling (Buchholz, F., *et al.*, *Nat. Biotechnol. 16*:657-662 (1998)), followed by selection of constructs that bind target proteins. This is repeated until target binding is achieved.

Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in medicine, genetics, molecular biology, biochemistry, pharmacology and/or related fields are intended to be within the scope of the following claims.

All publications and patents mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patents mentioned are herein incorporated by reference to the same extent as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications can be practiced within the scope of the appended claims.

Claims

What Is Claimed Is:

1. A therapeutic agent for combating Alzheimer's disease, wherein said agent can replace or supplement α_2M function, or suppress expression of *A2M-2*.

2. An anti-LRP-Aβ molecule comprising, an Aβ binding domain, and an LRP binding domain, or a pharmaceutically acceptable salt thereof.

3. The anti-LRP-Aβ molecule of claim 2, wherein said molecule is a peptide, or a pharmaceutically acceptable salt thereof.

4. An anti-LRP-Aβ peptide comprising:

(a) an Aβ binding domain comprising 10-50 contiguous residues of SEQ ID NO:6; and

(b) an LRP binding domain comprising 10-50 contiguous residues of SEQ ID NO:8, wherein said 10-50 contiguous residues of SEQ ID NO:8 encompass residues 1366-1392, or a pharmaceutically acceptable salt thereof.

5. An anti-LRP-Aβ peptide comprising:

(a) an Aβ binding domain having an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26; and

(b) an LRP binding domain having the amino acid sequence of SEQ ID NO:10, or a pharmaceutically acceptable salt thereof.

6. An anti-LRP-Aβ peptide comprising:

(a) an Aβ binding domain having an amino acid sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:16, SEQ

ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26; and

(b) an LRP binding domain comprising 10-50 contiguous residues of SEQ ID NO:8, or a pharmaceutically acceptable salt thereof.

7. The anti-LRP-Aβ peptide of claims 4, 5 or 6, wherein said Aβ binding domain is connected to said LRP binding domain by a peptide bond.

8. The anti-LRP-Aβ peptide of claims 4, 5 or 6, wherein said Aβ binding domain is connected to said LRP binding domain by a linker.

9. The anti-LRP-Aβ peptide of claim 8, wherein said linker is selected from the group consisting of a peptide, or polyethylene glycol.

10. The anti-LRP-Aβ peptide of claim 8, wherein said linker comprises 1-20 glycine residues.

11. A nucleic acid comprising a polynucleotide encoding the anti-LRP-Aβ peptide of claims 4, 5, or 6.

12. An anti-LRP-Aβ peptide comprising a polypeptide having the sequence of SEQ ID NO:14, or a pharmaceutically acceptable salt thereof.

13. An anti-LRP-Aβ peptide comprising:

(a) an Aβ binding domain having an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, and SEQ ID NO:26;

(b) an LRP binding domain having the amino acid sequence of SEQ ID NO:10; and

(c) a linker connecting said Aβ binding domain to said LRP binding domain.

14. A nucleic acid molecule comprising a nucleotide encoding the anti-LRP-Aβ peptide of claims 12 or 13.

15. A nucleic acid molecule encoding an anti-LRP-Aβ peptide comprising:

(a) a region encoding an Aβ binding domain, comprising 30-150 contiguous nucleotides of SEQ ID NO:5; and

(b) a region encoding an LRP binding domain comprising 30-150 contiguous nucleotides of SEQ ID NO:7.

16. A nucleic acid molecule encoding an anti-LRP-Aβ peptide comprising:

(a) a region encoding an Aβ binding domain having a nucleotide sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25; and

(b) a region encoding an LRP binding domain having the nucleotide sequence of SEQ ID NO:9.

17. A nucleic acid molecule encoding an anti-LRP-Aβ peptide comprising:

(a) a region encoding an Aβ binding domain having a nucleotide sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:25; and

(b) a region encoding an LRP binding domain comprising 30-150 contiguous nucleotides of SEQ ID NO:7.

18. The nucleic acid molecule of claims 15, 16, or 17, wherein said region encoding said Aβ binding domain is connected to said region encoding said LRP binding domain by a phosphodiester bond.

19. The nucleic acid molecule of claims 15, 16 or 17, wherein said region encoding said Aβ binding domain is connected to said region encoding said LRP binding domain by a nucleotide encoding 1-20 glycine residues.

20. A nucleic acid molecule comprising, a polynucleotide having at least 95% homology to the nucleic acid molecule of claims 15, 16, or 17.

21. A nucleic acid molecule comprising, a first polynucleotide that hybridizes to a second polynucleotide, wherein said second polynucleotide is complementary to the nucleic acid molecule of claims 15, 16, or 17.

22. The nucleic acid molecule of claim 21, wherein said first polynucleotide hybridizes to said second polynucleotide under conditions comprising:

(a) incubating overnight at 42°C in a solution consisting of 50% formamide, 5x SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and a 20 μg/ml denatured, sheared salmon sperm DNA; and

(b) washing at 65°C in a solution consisting of 0.1x SSC.

23. A nucleic acid molecule comprising a polynucleotide having the nucleotide sequence of SEQ ID NO:13.

24. A nucleic acid molecule comprising a polynucleotide having at least 95% identity to the nucleotide sequence of SEQ ID NO:13.

25. A nucleic acid molecule comprising a first polynucleotide that hybridizes to a second polynucleotide, wherein said second polynucleotide is complementary to the nucleotide sequence of SEQ ID NO:13.

26. The nucleic acid molecule of claim 25, wherein said first polynucleotide hybridizes to said second polynucleotide under conditions comprising:

(a) incubating overnight at 42°C in a solution consisting of 50% formamide, 5x SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and a 20 μg/ml denatured, sheared salmon sperm DNA; and

(b) washing at 65°C in a solution consisting of 0.1x SSC.

27. A pharmaceutical composition comprising an anti-LRP-Aβ molecule, and one or more pharmaceutically acceptable carriers.

28. A pharmaceutical composition comprising the anti-LRP-Aβ peptide of claims 4, 5, 6, or 13, or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable carriers.

29. A pharmaceutical composition comprising an anti-LRP-Aβ peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4 or SEQ ID NO:14, or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable carriers.

30. A method of combating Alzheimer's Disease in a subject comprising administering an anti-LRP-Aβ molecule.

31. The method of claim 30, wherein said anti-LRP-Aβ molecule is a peptide.

32. A method of combating Alzheimer's Disease in a subject comprising administering the anti-LRP-Aβ peptide of claims 4, 5, 6 or 13, or a pharmaceutically acceptable salt thereof.

33. A method of combating Alzheimer's Disease in a subject comprising administering an anti-LRP-Aβ peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:14, or a pharmaceutically acceptable salt thereof.

34. An *A2M-2* antisense oligonucleotide comprising a nucleotide designed to target *A2M-2* RNA.

35. The *A2M-2* antisense oligonucleotide of claim 34, wherein said RNA is hnRNA.

36. The *A2M-2* antisense oligonucleotide of claim 34, wherein said RNA is mRNA.

37. An *A2M-2* antisense oligonucleotide comprising a nucleotide having the sequence of SEQ ID NO:27.

38. An *A2M-2* antisense oligonucleotide comprising a nucleotide having the sequence of the last 15-30 contiguous nucleotides of SEQ ID NO:27.

39. An *A2M-2* antisense oligonucleotide comprising nucleotides 36 -50 of SEQ ID NO:27.

40. An *A2M-2* antisense oligonucleotide comprising nucleotides 20 -50 of SEQ ID NO:27.

41. A pharmaceutical composition comprising the *A2M-2* antisense oligonucleotide of claims 34, 35, 36, 37, 38, 39 or 40, and one or more pharmaceutically acceptable carriers.

42. A method of combating Alzheimer's Disease in a subject comprising administering the*A2M-2* antisense oligonucleotide of claims 34, 35, 36, 37, 38, 39 or 40.

43. A vector for gene therapy of Alzheimer's Disease, comprising a viral vector, wherein said viral vector carries a transgene selected from the group consisting of a gene encoding α_2M, and a gene encoding an anti-LRP-Aβ peptide.

44. The viral vector of claim 43, wherein said transgene is a gene encoding α_2M.

45. The viral vector of claim 44, wherein said transgene has the nucleotide sequence of nucleotides 44-4465 of SEQ ID NO:1.

46. The viral vector of claim 43, wherein said transgene is a gene encoding an anti-LRP-Aβ peptide.

47. The viral vector of claim 43, where in said transgene encodes the anti-LRP-AB peptide of claims 4, 5, 6, 12 or 13.

48. The viral vector of claims 43, 44, 45 or 46, wherein said viral vector is an adeno-associated virus.

49. A pharmaceutical composition comprising the viral vector of claims 43, 44, 45 or 46, and one or more pharmaceutically acceptable carriers.

50. A method of combating Alzheimer's Disease in a subject by administering the viral vector of claims 43, 44, 45 or 46.

51. A method of screening for a therapeutic agent for Alzheimer's Disease comprising the steps of:

(a) incubating cells in the presence of a test agent, wherein said cells are heterozygous or homozygous for the *A2M-2* allele, and wherein said cells express *A2M-2*; and

(b) determining whether the ratio of normal to aberrant *A2M* mRNA has increased relative to the ratio of normal to aberrant *A2M* mRNA found in cells untreated with test agent.

52. The method of claim 51, wherein said cells are glioma cells.

53. The method of claim 51, wherein said cells are hepatoma cells.

54. The method of claim 51, wherein said cells are heterozygous for the *A2M-2* allele.

55. The method of claim 51, wherein said cells are homozygous for the *A2M-2* allele.

56. The method of claim 51 wherein said step (b) comprises S1 nuclease analysis using a probe complementary to SEQ ID NO:1, wherein said probe encompasses nucleotides 2057-2284 of SEQ ID NO:1.

57. The method of claim 56, wherein said probe is 300 bp long.

58. The method of claim 51, wherein said step (b) comprises S1 nuclease analysis using a probe complementary to nucleotides 2024-2323 of SEQ ID NO:1.

59. The method of claim 51, wherein said step (b) comprises RT PCR analysis.

60. The method of claim 59, wherein said step (b) comprises RT PCR analysis using primers designed to amplify a region of *A2M* encompassing exons 17-18.

61. The method of claim 60, wherein said region of *A2M* encompassing exons 17-18 is 300 bp long.

62. The method of claim 60, wherein said primers are designed to amplify nucleotides 2052-2289 of SEQ ID NO:1.

63. The method of claim 60, wherein said primers consist of a first primer having a nucleotide sequence complementary to nucleotides 2024-2038 of SEQ ID NO:1, and a second primer having the nucleotide sequence of nucleotides 2309-2323 of SEQ ID NO:1.

64. A method of screening for a therapeutic agent for Alzheimer's Disease comprising the steps of:

(a) incubating α_2M with a test agent; and

(b) determining whether said α_2M of step (b) has undergone a conformational change; wherein said steps are performed in sequential order.

65. The method of claim 64, wherein said step (b) comprises performing an α_2M electrophoretic mobility assay.

66. A method of screening for a therapeutic agent for Alzheimer's Disease comprising the steps of:

(a) incubating α_2M with a test agent; and

(b) determining whether said α_2M of step (b) can bind to LRP; wherein said steps are performed in sequential order.

67. The method of claims 64, 65 or 66, wherein said α_2M is tetrameric.

68. The method of claim 66, wherein said step (b) comprises performing an ELISA.

69. The method of claim 68, wherein said ELISA comprises the steps of:

(a) incubating LRP in a well coated with anti-LRP IgG;

(b) incubating said well with said α_2M;

(c) incubating said well with anti-α_2M IgG conjugated to an enzyme; and

(d) incubating said well with a substrate for said enzyme; wherein said steps are performed in sequential order.

70. The method of claim 68, wherein said ELISA comprises the steps of:

(a) incubating a well coated with LRP with said α_2M;

(b) incubating said well with anti-α_2M IgG conjugated to an enzyme; and

(c) incubating said well with the substrate for said enzyme; wherein said steps are performed in sequential order.

71. The method of claim 68, wherein said ELISA comprises the steps of:

(a) incubating said α_2M in a well coated with an anti-α_2M IgG specific for activated α_2M;

(b) incubating said well with said α_2M;

(c) incubating said well with anti-α_2M IgG conjugated to an enzyme; and

(d) incubating said well with a substrate for said enzyme;

wherein said steps are performed in sequential order.

72. The method of claim 66, wherein said step (b) comprises immunoblotting.

73. The method of claim 72, wherein anti-LRP IgG and anti-α_2M IgG are used to perform said immunoblotting.

74. The method of claim 66, wherein said step (b) comprises determining the ability of said α_2M to undergo LRP mediated endocytosis.

75. The method of claim 66, wherein said step (b) comprises determining the ability of said α_2M to undergo LRP mediated degradation.

76. A nucleic acid molecule comprising a polynucleotide encoding the anti-LRP-Aβ peptide of claim 10.

77. A nucleic acid molecule comprising a polynucleotide having at least 95% homology to the nucleic acid molecule of claim 18.

78. A nucleic acid molecule comprising polynucleotide having at least 95% homology to the nucleic acid molecule of claim 19.

79. A nucleic acid molecule comprising a first polynucleotide that hybridizes to a second polynucleotide, wherein said second polynucleotide is complementary to the nucleic acid molecule of claim 18.

80. A nucleic acid molecule comprising a first polynucleotide that hybridizes to a second polynucleotide, wherein said second polynucleotide is complementary to the nucleic acid molecule of claim 19.

81. The viral vector of claim 47, wherein said viral vector is an adeno-associated virus.

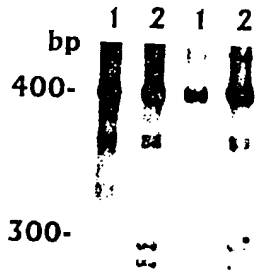

FIG. 1

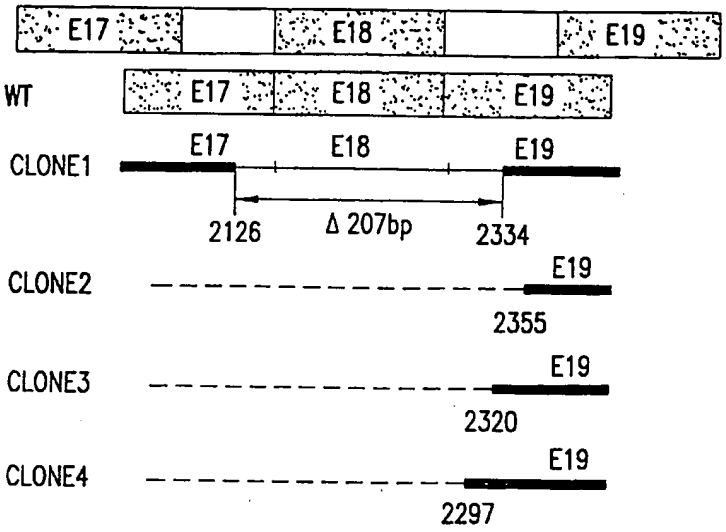
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FIG.2

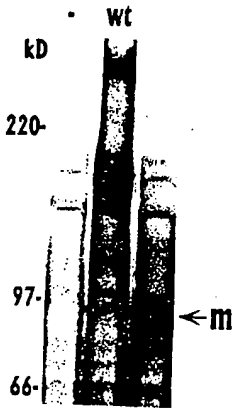

FIG.3A

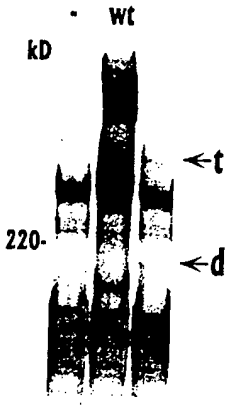

FIG.3B

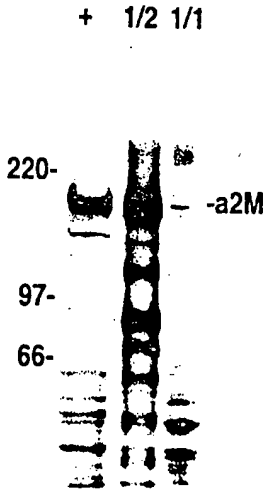

FIG.4

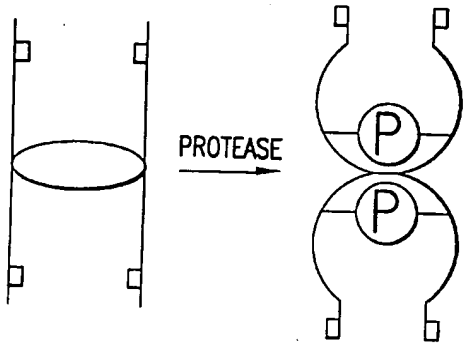

FIG.5

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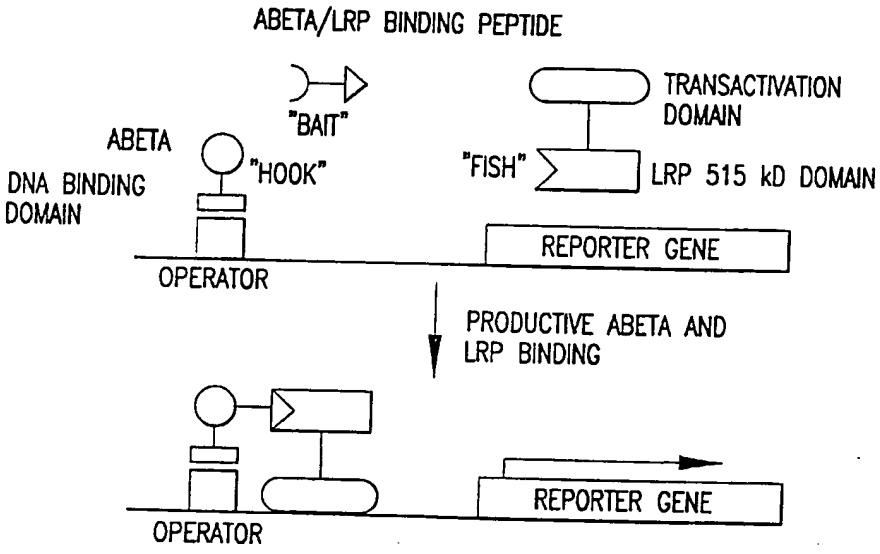
ANTI–Aβ ANTI–LRP

FIG.6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TRANSACTIVATION DOMAIN
ABETA
DNA BINDING DOMAIN
"HOOK"
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LRP 515 kD DOMAIN
REPORTER GENE
OPERATOR
PRODUCTIVE ABETA AND LRP BINDING
REPORTER GENE
OPERATOR

FIG.7

SEQUENCE LISTING

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55 Fruit Street
Boston, MA 02114

<120> Alpha-2-Macroglobulin Therapies and Drug Screening Methods for Alzheimer's Disease

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aca gtc cct gac acc atc acc gag tgg aag gca ggg gcc ttc tgc ctg 2359
Thr Val Pro Asp Thr Ile Thr Glu Trp Lys Ala Gly Ala Phe Cys Leu
735 740 745

tct gaa gat gct gga ctt ggt atc tct tcc act gcc tct ctc cga gcc 2407
Ser Glu Asp Ala Gly Leu Gly Ile Ser Ser Thr Ala Ser Leu Arg Ala
750 755 760 765

ttc cag ccc ttc ttt gtg gag ctt aca atg cct tac tct gtg att cgt 2455
Phe Gln Pro Phe Phe Val Glu Leu Thr Met Pro Tyr Ser Val Ile Arg
770 775 780

gga gag gcc ttc aca ctc aag gcc acg gtc cta aac tac ctt ccc aaa 2503
Gly Glu Ala Phe Thr Leu Lys Ala Thr Val Leu Asn Tyr Leu Pro Lys
785 790 795

tgc atc cgg gtc agt gtg cag ctg gaa gcc tct ccc gcc ttc ctt gct 2551

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Cys Ile Arg Val Ser Val Gln Leu Glu Ala Ser Pro Ala Phe Leu Ala
        800                 805                 810

gtc cca gtg gag aag gaa caa gcg cct cac tgc atc tgt gca aac ggg   2599
Val Pro Val Glu Lys Glu Gln Ala Pro His Cys Ile Cys Ala Asn Gly
    815                 820                 825

cgg caa act gtg tcc tgg gca gta acc cca aag tca tta gga aat gtg   2647
Arg Gln Thr Val Ser Trp Ala Val Thr Pro Lys Ser Leu Gly Asn Val
830                 835                 840                 845

aat ttc act gtg agc gca gag gca cta gag tct caa gag ctg tgt ggg   2695
Asn Phe Thr Val Ser Ala Glu Ala Leu Glu Ser Gln Glu Leu Cys Gly
                850                 855                 860

act gag gtg cct tca gtt cct gaa cac gga agg aaa gac aca gtc atc   2743
Thr Glu Val Pro Ser Val Pro Glu His Gly Arg Lys Asp Thr Val Ile
            865                 870                 875

aag cct ctg ttg gtt gaa cct gaa gga cta gag aag gaa aca aca ttc   2791
Lys Pro Leu Leu Val Glu Pro Glu Gly Leu Glu Lys Glu Thr Thr Phe
        880                 885                 890

aac tcc cta ctt tgt cca tca ggt ggt gag gtt tct gaa gaa tta tcc   2839
Asn Ser Leu Leu Cys Pro Ser Gly Gly Glu Val Ser Glu Glu Leu Ser
    895                 900                 905

ctg aaa ctg cca cca aat gtg gta gaa gaa tct gcc cga gct tct gtc   2887
Leu Lys Leu Pro Pro Asn Val Val Glu Glu Ser Ala Arg Ala Ser Val
910                 915                 920                 925

tca gtt ttg gga gac ata tta ggc tct gcc atg caa aac aca caa aat   2935
Ser Val Leu Gly Asp Ile Leu Gly Ser Ala Met Gln Asn Thr Gln Asn
                930                 935                 940

ctt ctc cag atg ccc tat ggc tgt gga gag cag aat atg gtc ctc ttt   2983
Leu Leu Gln Met Pro Tyr Gly Cys Gly Glu Gln Asn Met Val Leu Phe
            945                 950                 955

gct cct aac atc tat gta ctg gat tat cta aat gaa aca cag cag ctt   3031
Ala Pro Asn Ile Tyr Val Leu Asp Tyr Leu Asn Glu Thr Gln Gln Leu
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960 965 970

act cca gag atc aag tcc aag gcc att ggc tat ctc aac act ggt tac 3079
Thr Pro Glu Ile Lys Ser Lys Ala Ile Gly Tyr Leu Asn Thr Gly Tyr
975 980 985

cag aga cag ttg aac tac aaa cac tat gat ggc tcc tac agc acc ttt 3127
Gln Arg Gln Leu Asn Tyr Lys His Tyr Asp Gly Ser Tyr Ser Thr Phe
990 995 1000 1005

ggg gag cga tat ggc agg aac cag ggc aac acc tgg ctc aca gcc ttt 3175
Gly Glu Arg Tyr Gly Arg Asn Gln Gly Asn Thr Trp Leu Thr Ala Phe
1010 1015 1020

gtt ctg aag act ttt gcc caa gct cga gcc tac atc ttc atc gat gaa 3223
Val Leu Lys Thr Phe Ala Gln Ala Arg Ala Tyr Ile Phe Ile Asp Glu
1025 1030 1035

gca cac att acc caa gcc ctc ata tgg ctc tcc cag agg cag aag gac 3271
Ala His Ile Thr Gln Ala Leu Ile Trp Leu Ser Gln Arg Gln Lys Asp
1040 1045 1050

aat ggc tgt ttc agg agc tct ggg tca ctg ctc aac aat gcc ata aag 3319
Asn Gly Cys Phe Arg Ser Ser Gly Ser Leu Leu Asn Asn Ala Ile Lys
1055 1060 1065

gga gga gta gaa gat gaa gtg acc ctc tcc gcc tat atc acc atc gcc 3367
Gly Gly Val Glu Asp Glu Val Thr Leu Ser Ala Tyr Ile Thr Ile Ala
1070 1075 1080 1085

ctt ctg gag att cct ctc aca gtc act cac cct gtt gtc cgc aat gcc 3415
Leu Leu Glu Ile Pro Leu Thr Val Thr His Pro Val Val Arg Asn Ala
1090 1095 1100

ctg ttt tgc ctg gag tca gcc tgg aag aca gca caa gaa ggg gac cat 3463
Leu Phe Cys Leu Glu Ser Ala Trp Lys Thr Ala Gln Glu Gly Asp His
1105 1110 1115

ggc agc cat gta tat acc aaa gca ctg ctg gcc tat gct ttt gcc ctg 3511
Gly Ser His Val Tyr Thr Lys Ala Leu Leu Ala Tyr Ala Phe Ala Leu
1120 1125 1130

gca ggt aac cag gac aag agg aag gaa gta ctc aag tca ctt aat gag 3559
Ala Gly Asn Gln Asp Lys Arg Lys Glu Val Leu Lys Ser Leu Asn Glu
1135 1140 1145

gaa gct gtg aag aaa gac aac tct gtc cat tgg gag cgc cct cag aaa 3607
Glu Ala Val Lys Lys Asp Asn Ser Val His Trp Glu Arg Pro Gln Lys
1150 1155 1160 1165

ccc aag gca cca gtg ggg cat ttt tac gaa ccc cag gct ccc tct gct 3655
Pro Lys Ala Pro Val Gly His Phe Tyr Glu Pro Gln Ala Pro Ser Ala
1170 1175 1180

gag gtg gag atg aca tcc tat gtg ctc ctc gct tat ctc acg gcc cag 3703
Glu Val Glu Met Thr Ser Tyr Val Leu Leu Ala Tyr Leu Thr Ala Gln
1185 1190 1195

cca gcc cca acc tcg gag gac ctg acc tct gca acc aac atc gtg aag 3751
Pro Ala Pro Thr Ser Glu Asp Leu Thr Ser Ala Thr Asn Ile Val Lys
1200 1205 1210

tgg atc acg aag cag cag aat gcc cag ggc ggt ttc tcc tcc acc cag 3799
Trp Ile Thr Lys Gln Gln Asn Ala Gln Gly Gly Phe Ser Ser Thr Gln
1215 1220 1225

gac aca gtg gtg gct ctc cat gct ctg tcc aaa tat gga gcc gcc aca 3847
Asp Thr Val Val Ala Leu His Ala Leu Ser Lys Tyr Gly Ala Ala Thr
1230 1235 1240 1245

ttt acc agg act ggg aag gct gca cag gtg act atc cag tct tca ggg 3895
Phe Thr Arg Thr Gly Lys Ala Ala Gln Val Thr Ile Gln Ser Ser Gly
1250 1255 1260

aca ttt tcc agc aaa ttc caa gtg gac aac aac aat cgc ctg tta ctg 3943
Thr Phe Ser Ser Lys Phe Gln Val Asp Asn Asn Asn Arg Leu Leu Leu
1265 1270 1275

cag cag gtc tca ttg cca gag ctg cct ggg gaa tac agc atg aaa gtg 3991
Gln Gln Val Ser Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys Val
1280 1285 1290

aca gga gaa gga tgt gtc tac ctc cag acc tcc ttg aaa tac aat att 4039
Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn Ile
1295 1300 1305

ctc cca gaa aag gaa gag ttc ccc ttt gct tta gga gtg cag act ctg 4087
Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr Leu
1310 1315 1320 1325

cct caa act tgt gat gaa ccc aaa gcc cac acc agc ttc caa atc tcc 4135
Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser
1330 1335 1340

cta agt gtc agt tac aca ggg agc cgc tct gcc tcc aac atg gcg atc 4183
Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile
1345 1350 1355

gtt gat gtg aag atg gtc tct ggc ttc att ccc ctg aag cca aca gtg 4231
Val Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val
1360 1365 1370

aaa atg ctt gaa aga tct aac cat gtg agc cgg aca gaa gtc agc agc 4279
Lys Met Leu Glu Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser
1375 1380 1385

aac cat gtc ttg att tac ctt gat aag gtg tca aat cag aca ctg agc 4327
Asn His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln Thr Leu Ser
1390 1395 1400 1405

ttg ttc ttc acg gtt ctg caa gat gtc cca gta aga gat ctc aaa cca 4375
Leu Phe Phe Thr Val Leu Gln Asp Val Pro Val Arg Asp Leu Lys Pro
1410 1415 1420

gcc ata gtg aaa gtc tat gat tac tac gag acg gat gag ttt gca atc 4423
Ala Ile Val Lys Val Tyr Asp Tyr Tyr Glu Thr Asp Glu Phe Ala Ile
1425 1430 1435

gct gag tac aat gct cct tgc agc aaa gat ctt gga aat gct tga 4468
Ala Glu Tyr Asn Ala Pro Cys Ser Lys Asp Leu Gly Asn Ala
1440 1445 1450

agaccacaag gctgaaaagt gctttgctgg agtcctgttc tctgagctcc acagaagaca 4528

cgtgtttttg tatctttaaa gacttgatga ataaacactt tttctggtc 4577

<210> 2
<211> 1474
<212> PRT
<213> Homo sapiens

<400> 2

Met Gly Lys Asn Lys Leu Leu His Pro Ser Leu Val Leu Leu Leu Leu
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Val Leu Leu Pro Thr Asp Ala Ser Val Ser Gly Lys Pro Gln Tyr Met
20 25 30

Val Leu Val Pro Ser Leu Leu His Thr Glu Thr Thr Glu Lys Gly Cys
35 40 45

Val Leu Leu Ser Tyr Leu Asn Glu Thr Val Thr Val Ser Ala Ser Leu
50 55 60

Glu Ser Val Arg Gly Asn Arg Ser Leu Phe Thr Asp Leu Glu Ala Glu
65 70 75 80

Asn Asp Val Leu His Cys Val Ala Phe Ala Val Pro Lys Ser Ser Ser
85 90 95

Asn Glu Glu Val Met Phe Leu Thr Val Gln Val Lys Gly Pro Thr Gln
100 105 110

Glu Phe Lys Lys Arg Thr Thr Val Met Val Lys Asn Glu Asp Ser Leu
115 120 125

Val Phe Val Gln Thr Asp Lys Ser Ile Tyr Lys Pro Gly Gln Thr Val
130 135 140

Lys Phe Arg Val Val Ser Met Asp Glu Asn Phe His Pro Leu Asn Glu
145 150 155 160

Leu Ile Pro Leu Val Tyr Ile Gln Asp Pro Lys Gly Asn Arg Ile Ala

165 170 175

Gln Trp Gln Ser Phe Gln Leu Glu Gly Gly Leu Lys Gln Phe Ser Phe
180 185 190

Pro Leu Ser Ser Glu Pro Phe Gln Gly Ser Tyr Lys Val Val Val Gln
195 200 205

Lys Lys Ser Gly Gly Arg Thr Glu His Pro Phe Thr Val Glu Glu Phe
210 215 220

Val Leu Pro Lys Phe Glu Val Gln Val Thr Val Pro Lys Ile Ile Thr
225 230 235 240

Ile Leu Glu Glu Glu Met Asn Val Ser Val Cys Gly Leu Tyr Thr Tyr
245 250 255

Gly Lys Pro Val Pro Gly His Val Thr Val Ser Ile Cys Arg Lys Tyr
260 265 270

Ser Asp Ala Ser Asp Cys His Gly Glu Asp Ser Gln Ala Phe Cys Glu
275 280 285

Lys Phe Ser Gly Gln Leu Asn Ser His Gly Cys Phe Tyr Gln Gln Val
290 295 300

Lys Thr Lys Val Phe Gln Leu Lys Arg Lys Glu Tyr Glu Met Lys Leu
305 310 315 320

His Thr Glu Ala Gln Ile Gln Glu Glu Gly Thr Val Val Glu Leu Thr
325 330 335

Gly Arg Gln Ser Ser Glu Ile Thr Arg Thr Ile Thr Lys Leu Ser Phe
340 345 350

Val Lys Val Asp Ser His Phe Arg Gln Gly Ile Pro Phe Phe Gly Gln
355 360 365

Val Arg Leu Val Asp Gly Lys Gly Val Pro Ile Pro Asn Lys Val Ile
370 375 380

Phe Ile Arg Gly Asn Glu Ala Asn Tyr Tyr Ser Asn Ala Thr Thr Asp
385 390 395 400

Glu His Gly Leu Val Gln Phe Ser Ile Asn Thr Thr Asn Val Met Gly
405 410 415

Thr Ser Leu Thr Val Arg Val Asn Tyr Lys Asp Arg Ser Pro Cys Tyr
420 425 430

Gly Tyr Gln Trp Val Ser Glu Glu His Glu Glu Ala His His Thr Ala
435 440 445

Tyr Leu Val Phe Ser Pro Ser Lys Ser Phe Val His Leu Glu Pro Met
450 455 460

Ser His Glu Leu Pro Cys Gly His Thr Gln Thr Val Gln Ala His Tyr
465 470 475 480

Ile Leu Asn Gly Gly Thr Leu Leu Gly Leu Lys Lys Leu Ser Phe Tyr
485 490 495

Tyr Leu Ile Met Ala Lys Gly Gly Ile Val Arg Thr Gly Thr His Gly
500 505 510

Leu Leu Val Lys Gln Glu Asp Met Lys Gly His Phe Ser Ile Ser Ile
515 520 525

Pro Val Lys Ser Asp Ile Ala Pro Val Ala Arg Leu Leu Ile Tyr Ala
530 535 540

Val Leu Pro Thr Gly Asp Val Ile Gly Asp Ser Ala Lys Tyr Asp Val
545 550 555 560

Glu Asn Cys Leu Ala Asn Lys Val Asp Leu Ser Phe Ser Pro Ser Gln
565 570 575

Ser Leu Pro Ala Ser His Ala His Leu Arg Val Thr Ala Ala Pro Gln
580 585 590

Ser Val Cys Ala Leu Arg Ala Val Asp Gln Ser Val Leu Leu Met Lys
595 600 605

Pro Asp Ala Glu Leu Ser Ala Ser Ser Val Tyr Asn Leu Leu Pro Glu
610 615 620

Lys Asp Leu Thr Gly Phe Pro Gly Pro Leu Asn Asp Gln Asp Asp Glu
625 630 635 640

Asp Cys Ile Asn Arg His Asn Val Tyr Ile Asn Gly Ile Thr Tyr Thr
645 650 655

Pro Val Ser Ser Thr Asn Glu Lys Asp Met Tyr Ser Phe Leu Glu Asp
660 665 670

Met Gly Leu Lys Ala Phe Thr Asn Ser Lys Ile Arg Lys Pro Lys Met
675 680 685

Cys Pro Gln Leu Gln Gln Tyr Glu Met His Gly Pro Glu Gly Leu Arg
690 695 700

Val Gly Phe Tyr Glu Ser Asp Val Met Gly Arg Gly His Ala Arg Leu
705 710 715 720

Val His Val Glu Glu Pro His Thr Glu Thr Val Arg Lys Tyr Phe Pro
725 730 735

Glu Thr Trp Ile Trp Asp Leu Val Val Val Asn Ser Ala Gly Val Ala
740 745 750

Glu Val Gly Val Thr Val Pro Asp Thr Ile Thr Glu Trp Lys Ala Gly
755 760 765

Ala Phe Cys Leu Ser Glu Asp Ala Gly Leu Gly Ile Ser Ser Thr Ala
770 775 780

Ser Leu Arg Ala Phe Gln Pro Phe Phe Val Glu Leu Thr Met Pro Tyr
785 790 795 800

Ser Val Ile Arg Gly Glu Ala Phe Thr Leu Lys Ala Thr Val Leu Asn
805 810 815

Tyr Leu Pro Lys Cys Ile Arg Val Ser Val Gln Leu Glu Ala Ser Pro

820 825 830

Ala Phe Leu Ala Val Pro Val Glu Lys Glu Gln Ala Pro His Cys Ile
835 840 845

Cys Ala Asn Gly Arg Gln Thr Val Ser Trp Ala Val Thr Pro Lys Ser
850 855 860

Leu Gly Asn Val Asn Phe Thr Val Ser Ala Glu Ala Leu Glu Ser Gln
865 870 875 880

Glu Leu Cys Gly Thr Glu Val Pro Ser Val Pro Glu His Gly Arg Lys
885 890 895

Asp Thr Val Ile Lys Pro Leu Leu Val Glu Pro Glu Gly Leu Glu Lys
900 905 910

Glu Thr Thr Phe Asn Ser Leu Leu Cys Pro Ser Gly Gly Glu Val Ser
915 920 925

Glu Glu Leu Ser Leu Lys Leu Pro Pro Asn Val Val Glu Glu Ser Ala
930 935 940

Arg Ala Ser Val Ser Val Leu Gly Asp Ile Leu Gly Ser Ala Met Gln
945 950 955 960

Asn Thr Gln Asn Leu Leu Gln Met Pro Tyr Gly Cys Gly Glu Gln Asn
965 970 975

Met Val Leu Phe Ala Pro Asn Ile Tyr Val Leu Asp Tyr Leu Asn Glu
980 985 990

Thr Gln Gln Leu Thr Pro Glu Ile Lys Ser Lys Ala Ile Gly Tyr Leu
995 1000 1005

Asn Thr Gly Tyr Gln Arg Gln Leu Asn Tyr Lys His Tyr Asp Gly Ser
1010 1015 1020

Tyr Ser Thr Phe Gly Glu Arg Tyr Gly Arg Asn Gln Gly Asn Thr Trp
025 1030 1035 1040

Leu Thr Ala Phe Val Leu Lys Thr Phe Ala Gln Ala Arg Ala Tyr Ile
1045 1050 1055

Phe Ile Asp Glu Ala His Ile Thr Gln Ala Leu Ile Trp Leu Ser Gln
1060 1065 1070

Arg Gln Lys Asp Asn Gly Cys Phe Arg Ser Ser Gly Ser Leu Leu Asn
1075 1080 1085

Asn Ala Ile Lys Gly Gly Val Glu Asp Glu Val Thr Leu Ser Ala Tyr
1090 1095 1100

Ile Thr Ile Ala Leu Leu Glu Ile Pro Leu Thr Val Thr His Pro Val
105 1110 1115 1120

Val Arg Asn Ala Leu Phe Cys Leu Glu Ser Ala Trp Lys Thr Ala Gln
1125 1130 1135

Glu Gly Asp His Gly Ser His Val Tyr Thr Lys Ala Leu Leu Ala Tyr
1140 1145 1150

Ala Phe Ala Leu Ala Gly Asn Gln Asp Lys Arg Lys Glu Val Leu Lys
1155 1160 1165

Ser Leu Asn Glu Glu Ala Val Lys Lys Asp Asn Ser Val His Trp Glu
1170 1175 1180

Arg Pro Gln Lys Pro Lys Ala Pro Val Gly His Phe Tyr Glu Pro Gln
185 1190 1195 1200

Ala Pro Ser Ala Glu Val Glu Met Thr Ser Tyr Val Leu Leu Ala Tyr
1205 1210 1215

Leu Thr Ala Gln Pro Ala Pro Thr Ser Glu Asp Leu Thr Ser Ala Thr
1220 1225 1230

Asn Ile Val Lys Trp Ile Thr Lys Gln Gln Asn Ala Gln Gly Gly Phe
1235 1240 1245

Ser Ser Thr Gln Asp Thr Val Val Ala Leu His Ala Leu Ser Lys Tyr
1250 1255 1260

Gly Ala Ala Thr Phe Thr Arg Thr Gly Lys Ala Ala Gln Val Thr Ile
265 1270 1275 1280

Gln Ser Ser Gly Thr Phe Ser Ser Lys Phe Gln Val Asp Asn Asn Asn
1285 1290 1295

Arg Leu Leu Leu Gln Gln Val Ser Leu Pro Glu Leu Pro Gly Glu Tyr
1300 1305 1310

Ser Met Lys Val Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu
1315 1320 1325

Lys Tyr Asn Ile Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly
1330 1335 1340

Val Gln Thr Leu Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser
345 1350 1355 1360

Phe Gln Ile Ser Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser
1365 1370 1375

Asp Met Ala Ile Val Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu
1380 1385 1390

Lys Pro Thr Val Lys Met Leu Glu Arg Ser Asn His Val Ser Arg Thr
1395 1400 1405

Glu Val Ser Ser Asn His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn
1410 1415 1420

Gln Thr Leu Ser Leu Phe Phe Thr Val Leu Gln Asp Val Pro Val Arg
425 1430 1435 1440

Asp Leu Lys Pro Ala Ile Val Lys Val Tyr Asp Tyr Tyr Glu Thr Asp
1445 1450 1455

Glu Phe Ala Ile Ala Glu Tyr Asn Ala Pro Cys Ser Lys Asp Leu Gly
1460 1465 1470

Asn Ala

<210> 3
<211> 750
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(750)
<223> Aβ/LRP Binding Domain

<400> 3

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tcg gag gac ctg acc tct gca acc aac atc gtg aag tgg atc acg aag   48
Ser Glu Asp Leu Thr Ser Ala Thr Asn Ile Val Lys Trp Ile Thr Lys
 1               5                  10                  15

cag cag aat gcc cag ggc ggt ttc tcc tcc acc cag gac aca gtg gtg   96
Gln Gln Asn Ala Gln Gly Gly Phe Ser Ser Thr Gln Asp Thr Val Val
            20                  25                  30

gct ctc cat gcc ctg tcc aaa tat gga gcc gcc aca ttt acc agg act  144
Ala Leu His Ala Leu Ser Lys Tyr Gly Ala Ala Thr Phe Thr Arg Thr
        35                  40                  45

ggg aag gct gca cag gtg act atc cag tct tca ggg aca ttt tcc agc  192
Gly Lys Ala Ala Gln Val Thr Ile Gln Ser Ser Gly Thr Phe Ser Ser
    50                  55                  60

aaa ttc caa gtg gac aac aac aat cgc ctg tta ctg cag cag gtc tca  240
Lys Phe Gln Val Asp Asn Asn Asn Arg Leu Leu Leu Gln Gln Val Ser
65                  70                  75                  80

ttg cca gag ctg cct ggg gaa tac agc atg aaa gtg aca gga gaa gga  288
Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys Val Thr Gly Glu Gly
                85                  90                  95

tgt gtc tac ctc cag acc tcc ttg aaa tac aat att ctc cca gaa aag  336
Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn Ile Leu Pro Glu Lys
            100                 105                 110
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gaa gag ttc ccc ttt gct tta gga gtg cag act ctg cct caa act tgt 384
Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr Leu Pro Gln Thr Cys
115 120 125

gat gaa ccc aaa gcc cac acc agc ttc caa atc tcc cta agt gtc agt 432
Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser Leu Ser Val Ser
130 135 140

tac aca ggg agc cgc tct gcc tcc aac atg gcg atc gtt gat gtg aag 480
Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile Val Asp Val Lys
145 150 155 160

atg gtc tct ggc ttc att ccc ctg aag cca aca gtg aaa atg ctt gaa 528
Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val Lys Met Leu Glu
165 170 175

aga tct aac cat gtg agc cgg aca gaa gtc agc agc aac cat gtc ttg 576
Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser Asn His Val Leu
180 185 190

att tac ctt gat aag gtg tca aat cag aca ctg agc ttg ttc ttc acg 624
Ile Tyr Leu Asp Lys Val Ser Asn Gln Thr Leu Ser Leu Phe Phe Thr
195 200 205

gtt ctg caa gat gtc cca gta aga gat ctc aaa cca gcc ata gtg aaa 672
Val Leu Gln Asp Val Pro Val Arg Asp Leu Lys Pro Ala Ile Val Lys
210 215 220

gtc tat gat tac tac gag acg gat gag ttt gca atc gct gag tac aat 720
Val Tyr Asp Tyr Tyr Glu Thr Asp Glu Phe Ala Ile Ala Glu Tyr Asn
225 230 235 240

gct cct tgc agc aaa gat ctt gga aat gct 750
Ala Pro Cys Ser Lys Asp Leu Gly Asn Ala
245 250

<210> 4
<211> 250
<212> PRT
<213> Homo sapiens

<400> 4

Ser Glu Asp Leu Thr Ser Ala Thr Asn Ile Val Lys Trp Ile Thr Lys
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Gln Gln Asn Ala Gln Gly Gly Phe Ser Ser Thr Gln Asp Thr Val Val
20 25 30

Ala Leu His Ala Leu Ser Lys Tyr Gly Ala Ala Thr Phe Thr Arg Thr
35 40 45

Gly Lys Ala Ala Gln Val Thr Ile Gln Ser Ser Gly Thr Phe Ser Ser
50 55 60

Lys Phe Gln Val Asp Asn Asn Asn Arg Leu Leu Leu Gln Gln Val Ser
65 70 75 80

Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys Val Thr Gly Glu Gly
85 90 95

Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn Ile Leu Pro Glu Lys
100 105 110

Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr Leu Pro Gln Thr Cys
115 120 125

Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser Leu Ser Val Ser
130 135 140

Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile Val Asp Val Lys
145 150 155 160

Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val Lys Met Leu Glu
165 170 175

Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser Asn His Val Leu
180 185 190

Ile Tyr Leu Asp Lys Val Ser Asn Gln Thr Leu Ser Leu Phe Phe Thr
195 200 205

Val Leu Gln Asp Val Pro Val Arg Asp Leu Lys Pro Ala Ile Val Lys
210 215 220

Val Tyr Asp Tyr Tyr Glu Thr Asp Glu Phe Ala Ile Ala Glu Tyr Asn
225 230 235 240

Ala Pro Cys Ser Lys Asp Leu Gly Asn Ala
245 250

<210> 5
<211> 333
<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(333)
<223> Aß Binding Domain

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1 5 10 15

cag cag aac gcc cag ggc ggt ttc tcc tcc acc cag gac aca gtg gtg 96
Gln Gln Asn Ala Gln Gly Gly Phe Ser Ser Thr Gln Asp Thr Val Val
20 25 30

gct ctc cat gct ctg tcc aaa tat gga gcc gcc aca ttt acc agg act 144
Ala Leu His Ala Leu Ser Lys Tyr Gly Ala Ala Thr Phe Thr Arg Thr
35 40 45

ggg aag gct gca cag gtg act atc cag tct tca ggg aca ttt tcc agc 192
Gly Lys Ala Ala Gln Val Thr Ile Gln Ser Ser Gly Thr Phe Ser Ser
50 55 60

aaa ttc caa gtg gac aac aac aat cgc ctg tta ctg cag cag gtc tca 240
Lys Phe Gln Val Asp Asn Asn Asn Arg Leu Leu Gln Gln Val Ser
65 70 75 80

ttg cca gag ctg cct ggg gaa tac agc atg aaa gtg aca gga gaa gga 288
Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys Val Thr Gly Glu Gly
85 90 95

tgt gtc tac ctc cag acc tcc ttg aaa tac aat att ctc cca gaa 333
Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn Ile Leu Pro Glu
100 105 110

<210> 6
<211> 111
<212> PRT
<213> Homo sapiens

<400> 6
Ser Glu Asp Leu Thr Ser Ala Thr Asn Ile Val Lys Trp Ile Thr Lys
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Gln Gln Asn Ala Gln Gly Gly Phe Ser Ser Thr Gln Asp Thr Val Val
20 25 30

Ala Leu His Ala Leu Ser Lys Tyr Gly Ala Ala Thr Phe Thr Arg Thr
35 40 45

Gly Lys Ala Ala Gln Val Thr Ile Gln Ser Ser Gly Thr Phe Ser Ser
50 55 60

Lys Phe Gln Val Asp Asn Asn Asn Arg Leu Leu Leu Gln Gln Val Ser
65 70 75 80

Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys Val Thr Gly Glu Gly
85 90 95

Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn Ile Leu Pro Glu
100 105 110

<210> 7
<211> 417
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(417)
<223> LRP Binding Domain

<400> 7

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aag gaa gag ttc ccc ttt gct tta gga gtg cag act ctg cct caa act   48
Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr Leu Pro Gln Thr
 1               5                  10                  15

tgt gat gaa ccc aaa gcc cac acc agc ttc caa atc tcc cta agt gtc   96
Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser Leu Ser Val
            20                  25                  30

agt tac aca ggg agc cgc tct gcc tcc aac atg gcg atc gtt gat gtg   144
Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile Val Asp Val
        35                  40                  45

aag atg gtc tct ggc ttc att ccc ctg aag cca aca gtg aaa atg ctt   192
Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val Lys Met Leu
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gaa aga tct aac cat gtg agc cgg aca gaa gtc agc agc aac cat gtc   240
Glu Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser Asn His Val
65                  70                  75                  80

ttg att tac ctt gat aag gtg tca aat cag aca ctg agc ttg ttc ttc   288
Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln Thr Leu Ser Leu Phe Phe
                85                  90                  95

acg gtt ctg caa gat gtc cca gta aga gat ctc aaa cca gcc ata gtg   336
Thr Val Leu Gln Asp Val Pro Val Arg Asp Leu Lys Pro Ala Ile Val
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aaa gtc tat gat tac tac gag acg gat gag ttt gca atc gct gag tac   384
Lys Val Tyr Asp Tyr Tyr Glu Thr Asp Glu Phe Ala Ile Ala Glu Tyr
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130 135

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Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile Val Asp Val
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Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val Lys Met Leu
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Glu Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser Asn His Val
65 70 75 80

Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln Thr Leu Ser Leu Phe Phe
85 90 95

Thr Val Leu Gln Asp Val Pro Val Arg Asp Leu Lys Pro Ala Ile Val
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Lys Val Tyr Asp Tyr Tyr Glu Thr Asp Glu Phe Ala Ile Ala Glu Tyr
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cca aca gtg aaa atg ctt gaa aga tct aac cat gtg agc cgg aca gaa 96
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INTERNATIONAL SEARCH REPORT

Inte. .onal Application No
PCT/US 00/02412

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/47 C12N15/12 C12N15/11 C12Q1/68 A61K48/00
C12N15/86 G01N33/53 A61K38/17 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2000	06/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mateo Rosell, A.M.

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INTERNATIONAL SEARCH REPORT

Inte national Application No
PCT/US 00/02412

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Category *	Citation of document, with indication,where appropriate, of the re evant passages	Relevant to claim No.
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X	WO 91 03557 A (NOVONORDISK AS) 21 March 1991 (1991-03-21) page 3, line 22-37 page 10, line 32 -page 13, line 27; examples 1,2,4	4,5,12, 13,16, 23-25, 27,28, 30-33, 37-40, 45,49, 50,56, 58,62, 63, 66-71, 77,80
X	WO 96 39834 A (UNIV NEW YORK) 19 December 1996 (1996-12-19) page 8, line 19 -page 9, line 11; example 1 SEQ.ID.N.8	5,6, 12-17, 23-25, 27,28, 30-33
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International Application No
PCT/US 00/02412

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	NIELSEN KARE L ET AL: "Identification of residues in alpha-macroglobulins important for binding to the alpha-2-macroglobulin receptor/low density lipoprotein receptor-related protein." JOURNAL OF BIOLOGICAL CHEMISTRY 1996, vol. 271, no. 22, 1996, pages 12909-12912, XP002140605 ISSN: 0021-9258 cited in the application the whole document	11-13
A	WO 92 03474 A (HARVARD COLLEGE) 5 March 1992 (1992-03-05) page 4, line 25 -page 14, line 27	1-3
A	POLLER W ET AL: "SEQUENCE POLYMORPHISM IN THE HUMAN ALPHA-2-MACROGLOBULIN (A2M) GENE" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 19, 1991, page 198 XP002915261 ISSN: 0305-1048 the whole document	1,37-40, 56,58-63
A	BLACKER D ET AL: "ALPHA-2 MACROGLOBULIN IS GENETICALLY ASSOCIATED WITH ALZHEIMER DISEASE" NATURE GENETICS,US,NEW YORK, NY, vol. 19, 19 August 1998 (1998-08-19), pages 357-360, XP002915257 ISSN: 1061-4036 cited in the application the whole document	1,56, 58-63

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INTERNATIONAL SEARCH REPORT

Inter .onal Application No
PCT/US 00/02412

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SOTO CLAUDIO ET AL: "beta-Sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: Implications for Alzheimer's therapy." NATURE MEDICINE JULY, 1998, vol. 4, no. 7, July 1998 (1998-07), pages 822-826, XP000914747 ISSN: 1078-8956 cited in the application the whole document	1,2,27, 28
A	JUSTUS C W E ET AL: "QUANTIFICATION OF FREE ALPHA2-MACROGLOBULIN AND ALPHA2-MACROGLOBULIN-PROTEASE COMPLEXES BY A NOVEL ELISA SYSTEM BASED ON STREPTOCOCCAL ALPHA2-MACROGLOBULIN RECEPTORS" JOURNAL OF IMMUNOLOGICAL METHODS,NL,ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, vol. 126, 24 January 1990 (1990-01-24), pages 103-108, XP002915262 ISSN: 0022-1759 the whole document	1,66-71
P,X	WO 99 11824 A (HYMAN BRADLEY T ;REBECK GEORGE W (US); TANZI RUDOLPH E (US)) 11 March 1999 (1999-03-11) the whole document	1,56-71

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/02412

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		JP 5500005 T	14-01-1993
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